(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 February 2003 (27.02.2003)

PCT

(10) International Publication Number
WO 03/015777 A1

(51) International Patent Classification[7]: A61K 31/426; C07D 277/40, 277/42, 277/44, 277/46

(21) International Application Number: PCT/US02/25438

(22) International Filing Date: 13 August 2002 (13.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01119473.5 13 August 2001 (13.08.2001) EP
10/185,731 1 July 2002 (01.07.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/015777 A1

(54) Title: NR1H4 NUCLEAR RECEPTOR BINDING COMPOUNDS

(57) Abstract: The present invention relates to compounds according to the general formula (I) which bind to the NR1H4 receptor and act as agonists of the NR1H4 receptor. The invention further relates to the treatment of diseases and/or conditions through binding of the nuclear receptor by the compounds and the production of medicaments using the compounds.

NR1H4 NUCLEAR RECEPTOR BINDING COMPOUNDS

Field of the Invention

[0001] The present invention relates to compounds that bind to the NR1H4 nuclear receptor and methods of treating diseases or pathological conditions influenced by NR1H4.

Background of the Invention

[0002] Multicellular organisms are dependent on advanced mechanisms of information transfer between cells and body compartments. The information that is transmitted can be highly complex and can result in the alteration of genetic programs involved in cellular differentiation, proliferation, or reproduction. The signals, or hormones, are often simple molecules, such as peptides, fatty acid, or cholesterol derivatives.

[0003] Many of these signals produce their effects by ultimately changing the transcription of specific genes. One well-studied group of proteins that mediate a cell's response to a variety of signals is the family of transcription factors known as nuclear receptors, hereinafter referred to often as "NR". Members of this group include receptors for steroid hormones, vitamin D, ecdysone, cis and trans retinoic acid, thyroid hormone, bile acids, cholesterol-derivatives, fatty acids (and other peroxisomal proliferators), as well as so-called orphan receptors, proteins that are structurally similar to other members of this group, but for which no ligands are known (Escriva, H. et al., Ligand binding was acquired during evolution of nuclear receptors, PNAS, 94, 6803-6808, 1997). Orphan receptors may be indicative of unknown signaling pathways in the cell or may be nuclear receptors that function without ligand activation. The activation of transcription by some of these orphan receptors may occur in the absence of an exogenous ligand and/or through signal transduction pathways originating from the cell surface (Mangelsdorf, D. J. et al., The nuclear receptor superfamily: the second decade, Cell 83, 835-839, 1995).

[0004] In general, three functional domains have been defined in NRs. An amino terminal domain is believed to have some regulatory function. A DNA-binding domain hereinafter referred to as "DBD" usually comprises two zinc finger elements and recognizes a specific Hormone Responsive Element (hereinafter referred to as "HRE") within the promoters of responsive genes. Specific amino acid residues in the "DBD" have been shown to confer DNA sequence binding specificity (Schena, M. & Yamamoto, K.R., Mammalian Glucocorticoid Receptor Derivatives Enhance Transcription in Yeast, Science, 241:965-967, 1988). A ligand-binding domain (hereinafter referred to as "LBD") is at the carboxy-terminal region of known NRs. In the absence of hormone, the LBD appears to interfere with the interaction of the DBD with its HRE. Hormone binding seems to result in a conformational change in the NR, and thus, opens this interference (Brzozowski et al., Molecular basis of agonism and antagonism in the oestogen receptor, Nature, 389, 753-758, 1997; Wagner et al., A structural role for hormone in the thyroid hormone receptor, Nature, 378, 690 – 697. 1995). A NR without the LBD constitutively activates transcription, but at a low level.

[0005] Coactivators or transcriptional activators are proposed to bridge between sequence specific transcription factors, the basal transcription machinery, and in addition, to influence the chromatin structure of a target cell. Several proteins like SRC-1, ACTR, and Grip1 interact with NRs in a ligand enhanced manner (Heery et al., A signature motif in transcriptional coactivators mediates binding to nuclear receptors, Nature, 387, 733-736; Heinzel et al., A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression, Nature 387, 43-47, 1997). Furthermore, the physical interaction with negative receptor-interacting proteins or corepressors has been demonstrated (Xu et al., Coactivator and Corepressor complexes in nuclear receptor function, Curr Opin Genet Dev, 9 (2), 140-147, 1999).

[0006] Nuclear receptor modulators like steroid hormones affect the growth and function of specific cells by binding to intracellular receptors and forming nuclear receptor-ligand complexes. Nuclear receptor-hormone complexes then interact with a HRE in the control region of specific genes and alter specific gene expression.

[0007] The Farnesoid X Receptor alpha (hereinafter to as "FXR" and also often referred to as "NR1H4" when referring to the human receptor) is a prototypical type 2 nuclear receptor which activates genes upon binding to promoter region of target genes in a heterodimeric fashion with Retinoid X Receptor (hereinafter referred to as "RXR") (Forman et al., Cell, 81, 687-93, 1995). The relevant physiological ligands of NR1H4 seem to be bile acids (Makishima et al., Science, 284, 1362-65, 1999; Parks et al., Science, 284, 1365-68, 1999). The most potent is chenodeoxycholic acid, which regulates the expression of several genes that participate in bile acid homeostasis. Farnesoid, originally described to activate the rat ortholog at high concentration does not activate the human or mouse receptor. FXR is expressed in the liver, small intestine, colon, ovary, adrenal gland and kidney. Like FXRα, NR1H4 is involved in intracrine signaling.

[0008] FXR is proposed to be a nuclear bile acid sensor. As a result, it modulates both, the synthetic output of bile acids in the liver and their recycling in the intestine (by regulating bile acid binding proteins). Upon activation (e.g. binding of chenodeoxycholic acid) it influences the conversion of dietary cholesterol into bile acids by inhibiting the transcription of key genes which are involved in bile acid synthesis such as CYP7A1. This seems to be a major mechanism of feedback regulation onto bile acid synthesis.

[0009] The synthetic compounds, 1,1-bisphosphonate esters, appear to display a number of similar activities to the two identified prototypes of natural FXR agonists, farnesol, and chenodeoxycholc acid. Like farnesol, the 1,1-bisphosphonate esters increase the rate of 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase degradation and like bile acids they induce the expression of the intestinal bile acid binding protein (hereinafter referred to as "I-BABP") and repress the cholesterol 7 α-hydroxylase gene. Certain 1,1-bisphosphonate esters also bind to FXR (Niesor et al., Curr Pharm Des,7(4):231-59, 2001). That means that activation of FXR could lead to opposing effects (lowering the rate of cholesterol synthesis by increasing degradation of HMG-CoA reductase and increasing the cholesterol pool by inhibition of cholesterol degradation into bile acids). The FXR

agonist, chenodeoxycholic acid, does not change cholesterol and lipoprotein levels significantly in patients, although a repression of bile acid synthesis as well as a decreased HMG-CoA reductase activity was observed (Einarsson et al., Hepatology, 33(5), 1189-93, 2001) confirming that cellular cholesterol synthesis and degradation are controlled by numerous regulatory loops including the coordinate regulation of HMGCoA reductase and cholesterol 7α-hydroxylase and that compounds modulating FXR acitvity might have different effects on blood lipid parameters.

[0010] In the course of functional analysis of certain 1,1-bisphosphonate esters, it was shown that these compounds which are known to bind to FXR also induce apoptosis in a variety of cell types, similar to the isporenoids farnesol and geranylgeraniol which are also known as weak FXR binders (Flach et al., Biochem Biophys Res Com, 270, 240-46, 2000).

[0011] To date only very few compounds have been described which bind the NR1H4 receptor, and thus, show utility for treating diseases or conditions which are due to or influenced by this nuclear receptor (Maloney at al., J Med Chem, 10; 43(16): 2971-2974, 2000).

[0012] It is an object of the present invention to provide for a novel NR1H4 binding compound. It was also an object of the present invention to provide for compounds which, by means of binding the NR1H4 receptor, act as an agonist or antagonist of said receptor, and thus, show utility for treating diseases or conditions which are due to or influenced by said nuclear receptor.

[0013] It is a further object of the invention to provide for compounds which may be used for the manufacture of a medicament for the treatment of cholesterol associated conditions or diseases. In a preferred embodiment of the invention it was an object of the invention to provide for cholesterol lowering or cholestatic compounds. It was also an object of the invention to provide for compounds may be used for the manufacture of antitumor medicaments.

Summary of the Invention

[0014] The present invention provides, *inter alia*, novel NR1H4 nuclear receptor protein binding compounds according to the general formula (I) shown below. These compounds are also binders of mammalian homologues of the receptor. Further the object of the invention is solved by providing for, amongst the NR1H4 nuclear receptor protein binding, compounds according to the general formula (I) which act as agonists and compounds which act as antagonists of the human FXR receptor or a mammalian homologue thereof.

[0015] The invention provides for FXR agonists which may be used for the treatment of cholesterol associated conditions or diseases. In a preferred embodiment of the invention cholesterol lowering or cholestatic compounds are disclosed. The compounds according to the invention may be used for manufacture of antitumor medicaments and/or for the treatment of diseases such as cancer.

[0016] The foregoing merely summarizes certain aspects of the present invention and is not intended, nor should it be construed, to limit the invention in any manner. All patents and other publications recited herein are hereby incorporated by reference in their entirety.

Brief Description of the Drawings

[0017] The various novel features of this invention, along with the foregoing and other objects as well as the invention itself may be more fully understood from the following description when read in conjunction with the accompanying drawings.

[0018] Figs. 1A and 1B show the synthesis of the compounds of the invention described in Example 2.

[0019] Fig. 2 shows the synthesis of the compounds of the invention described in Example 3.

[0020] Fig. 3 shows the synthesis of the compounds of the invention described in Example 4.

[0021] Fig. 4A shows SEQ ID No. 1 which is a protein sequence of the FRX protein, a portion of which can be used for cloning.

[0022] Fig. 4B shows SEQ ID NO. 2 which is the mRNA sequence encoding the FRX protein.

[0023} Fig. 4C shows SEQ ID NO. 3 which shows the protein sequence of TIF2 (Acc. No.: XM 011633 RefSeq DB).

[0024] Fig. 4D shows SEQ ID NO. 4 which is respective mRNA sequence corresponding to the TIF2 protein.

[0025] Fig 5 A shows a dose response with LN6348 in the HEK293-FXR reporter assay

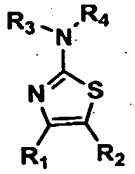
[0026] Fig 5B shows a dose response with LN6316 in the HEK293-FXR reporter assay

[0027] Fig 5C shows a dose response with LN6365 in the HEK293-FXR reporter assay

[0028] Fig 5D shows a dose response with LN6322 in the HEK293-FXR reporter assay

Detailed Description of the Invention

[0025] The invention provides for a compound including resolved diastereoisomers and enantiomers, and tautomers, pharmaceutical acceptable salts or solvates thereof (hereinafter also referred to as the "compounds according to the invention"), having the following formula (I):



(I)

wherein

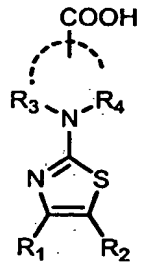
[0026] R_1 and R_2 are independently selected from the group consisting of C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, phenyl, substituted phenyl, C_7 to C_{12} phenylalkyl, C_7 to C_{12} substituted phenylalkyl, heterocyclic ring, substituted heterocyclic ring, heteroaryl, and substituted heteroaryl; and

[0027] R_3 and R_4 are independently selected from the group consisting of hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, phenyl, substituted phenyl, C_7 to C_{12} phenylalkyl, C_7 to C_{12} substituted phenylalkyl, naphthyl, substituted naphthyl, C_1 to C_8 alkanesulfonyl, C_1 to C_8 substituted alkanesulfonyl, benzenesulfonyl, substituted benzenesulfonyl, C_1 to C_8 acyl, and C_1 to C_8 substituted acyl; where R_3 and R_4 may be taken together with nitrogen to form a heterocycle or substituted heterocycle or a heteroaryl or substituted heteroaryl ring.

[0028] In one embodiment of the present invention, R_1 and R_2 in formula (I) are independently selected from the group consisting of phenyl, substituted phenyl, C_7 to C_{12} phenylalkyl, C_7 to C_{12} substituted phenylalkyl, heteroaryl, and substituted heteroaryl; R_3 and R_4 are independently selected from the group consisting of hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, substituted phenyl, C_7 to C_{12} phenylalkyl, C_7 to C_{12} substituted phenylalkyl, substituted naphthyl, C_1 to C_8 substituted alkanesulfonyl, and substituted benzenesulfonyl; where R_3 and R_4 may be taken together with nitrogen to form a heterocycle or a substituted heterocycle or a heteroaryl or a substituted heteroaryl ring.

[0029] In a more preferred embodiment of the present invention, R_1 and R_2 are independently selected from the group consisting of phenyl, substituted phenyl,

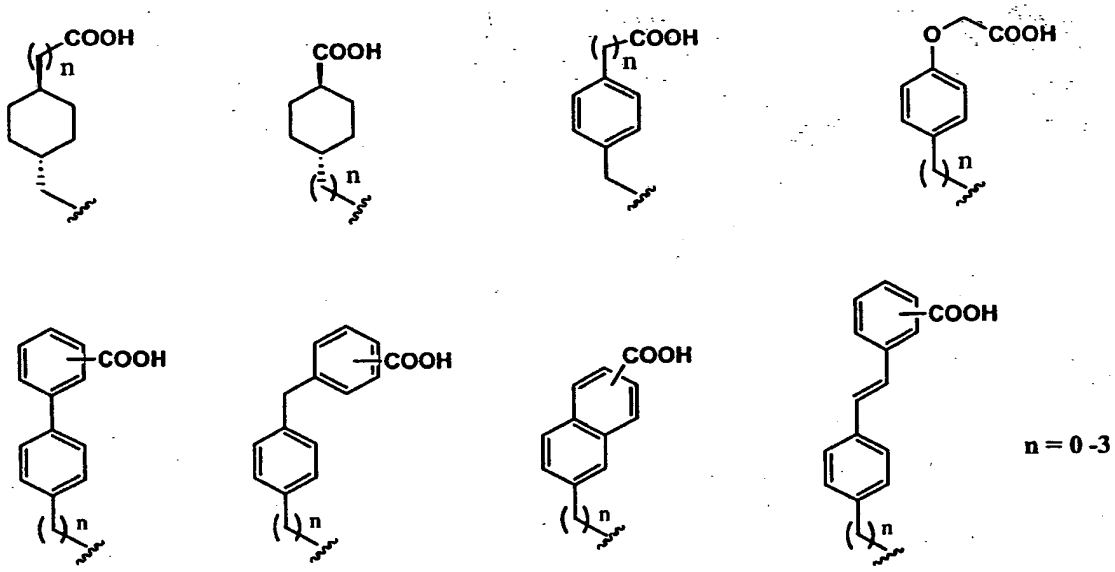
C_7 to C_{12} phenylalkyl, C_7 to C_{12} substituted phenylalkyl, heteroaryl, and substituted heteroaryl; R_3 and R_4 are independently selected from the group consisting of hydrogen, C_1 to C_8 substituted alkyl, substituted phenyl, C_7 to C_{12} substituted phenylalkyl, substituted naphthyl, C_1 to C_8 substituted alkanesulfonyl, and substituted benzenesulfonyl, wherein at least one of the mentioned above groups is substituted with carboxylic acid functionality as shown in formula (II) below:



(II)

where R_3 and R_4 may be taken together with nitrogen to form a heterocycle or a substituted heterocycle or a heteroaryl or substituted heteroaryl ring, also substituted with carboxylic acid functionality.

[0030] In a more preferred embodiment of the invention compounds are claimed, or pharmaceutical acceptable salts or solvates thereof, wherein R_1 and R_2 are independently selected from the group consisting of phenyl, substituted phenyl, C_7 to C_{12} phenylalkyl, C_7 to C_{12} substituted phenylalkyl, heteroaryl, and substituted heteroaryl; R_3 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 alkanesulfonyl, C_1 to C_8 substituted alkanesulfonyl, benzenesulfonyl, and substituted benzenesulfonyl; and R_4 is one of the following structural formulas:



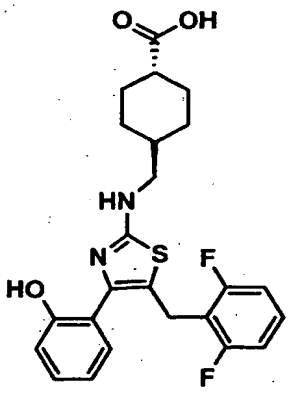
wherein n is an integer from 0 to 3. The symbol:

indicates the point of attachment of R_4 where R_4 is covalently bonded to Formula (I).

[0031] In a more preferred embodiment of the invention, there are provided compounds including resolved diastereoisomers and enantiomers, and tautomers, pharmaceutical acceptable salts or solvates thereof, wherein R_1 and R_2 are independently selected from the group consisting of substituted phenyl, C_7 to C_{12} substituted phenylalkyl, and substituted heteroaryl, where preferred substituents are taken from hydrogen, halogen, hydroxy or alkoxy groups; R_3 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 acyl, and C_1 to C_8 substituted acyl; and R_4 is one of the structures set forth above.

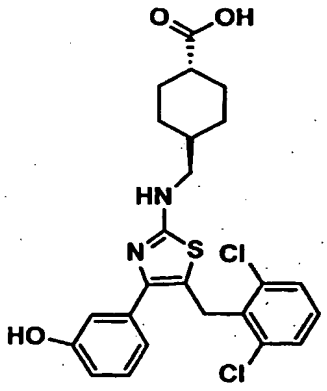
[0032] A particularly preferred compound which may act as agonist of NR1H4 is shown in formula (III) below. The inventors have been able to demonstrate that the compound according to formula (III) has a low effective binding concentration at FXR with an EC_{50} of 0.2 μM wherein the EC_{50} reflects the half-maximal effective

concentration, and which is higher than the EC_{50} of 0.015 μM for the published FXR agonist GW4064 (B.Goodwin et al., Molecular Cell 6, 517-526, 2000).

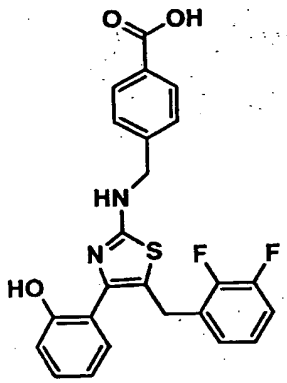


(III)

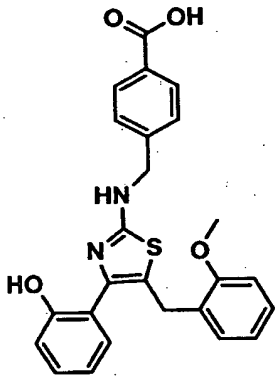
[0033] The inventors have also found the compounds according to formulas (IV), (V) and (VI) shown below to be active as agonist of the NR1H4 human nuclear receptor (see figures for details).



(IV)



(V)



(VI)

[0034] The inventors have identified the compounds as well as the general structure capable of effectively binding FXR.

[0035] The compounds of the invention can also exist as solvates and hydrates. Thus, these compounds may crystallize with, for example, waters of hydration, or one, a number of, or any fraction thereof of molecules of the mother liquor solvent. The solvates and hydrates of such compounds are included within the scope of this invention.

[0036] A solid carrier can be one or more substances which can also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

[0037] In powders, the carrier is generally a finely divided solid, which is in a mixture with the finely divided active component. In tablets, the active compound or combination of active compounds is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

[0038] For preparing a pharmaceutical composition in the form of suppositories, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient or combination of active ingredients is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient-sized molds and allowed to cool and solidify.

[0039] Powders and tablets preferably contain between about 5% to about 70% by weight of the active ingredient. Suitable carriers include, for example, magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter and the like.

[0040] The pharmaceutical compositions can include the formulation of the active compound(s) with encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is surrounded by a carrier, which is thus in association with it. In a similar manner, cachets are also included. Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral administration.

[0041] Liquid pharmaceutical compositions include, for example, solutions suitable for oral or parenteral administration, or suspensions, and emulsions suitable for oral administration. Sterile water solutions of the active component or sterile

solutions of the active component in solvents comprising water, ethanol, or propylene glycol are examples of liquid compositions suitable for parenteral administration.

[0042] Sterile solutions can be prepared by dissolving the active component(s) in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by dissolving the sterile compound in a previously sterilized solvent under sterile conditions.

[0043] The term "halogen" refers to the fluoro, chloro, bromo or iodo atoms. There can be one or more halogen, which are the same or different. Preferred halogens are chloro and fluoro.

[0044] The term "C_1 to C_8 alkyl" denotes such radicals as methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, amyl, tert-amyl, hexyl , n-heptyl, 2-heptyl, 3-heptyl, 4-heptyl, 2-methyl-1-hexyl, 2-methyl-2-hexyl, 2-methyl-3-hexyl, n-octyl ,and the like.

[0045] The term "C_1 to C_8 substituted alkyl" denotes that the above C_1 to C_8 alkyl groups are substituted by one or more, and preferably one or two, halogen, hydroxy, protected hydroxy, oxo, protected oxo, C_3 to C_7 cycloalkyl, phenyl, naphthyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, guanidino, protected guanidino, heterocyclic ring, substituted heterocyclic ring, C_1 to C_8 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, nitro, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N,N-di(C_1 to C_6 alkyl)carboxamide, cyano, methylsulfonylamino, thiol, C_1 to C_4 alkylthio or C_1 to C_4 alkylsulfonyl groups. The substituted alkyl groups may be substituted once or more, and preferably once or twice, with the same or with different substituents.

[0046] Examples of the above substituted alkyl groups include the 2-oxo-prop-1-yl, 3-oxo-but-1-yl, cyanomethyl, nitromethyl, chloromethyl, hydroxymethyl, tetrahydropyranyloxymethyl, trityloxymethyl, propionyloxymethyl, amino, methylamino, aminomethyl, dimethylamino, carboxymethyl, allyloxycarbonylmethyl,

allyloxycarbonylaminomethyl, methoxymethyl, ethoxymethyl, t-butoxymethyl, acetoxymethyl, 4-carboxybutyl, 5-carboxypentyl, 6-carboxyhexyl, chloromethyl, bromomethyl, iodomethyl, trifluoromethyl, 6-hydroxyhexyl, 2,4-dichloro(n-butyl), 2-aminopropyl, 1-chloroethyl, 2-chloroethyl, 1- bromoethyl, 2-chloroethyl, 1-fluoroethyl, 2-fluoroethyl, 1- iodoethyl, 2-iodoethyl, 1-chloropropyl, 2-chloropropyl, 3-chloropropyl, 1-bromopropyl, 2-bromopropyl, 3-bromopropyl, 1-fluoropropyl, 2-fluoropropyl, 3-fluoropropyl, 1- iodopropyl, 2-iodopropyl, 3-iodopropyl, 2-aminoethyl, 1- aminoethyl, N-benzoyl-2-aminoethyl, N-acetyl-2-aminoethyl, N-benzoyl-1-aminoethyl, N-acetyl-1-aminoethyl, and the like.

[0047] The term "substituted phenyl" specifies a phenyl group substituted with one or more, and preferably one or two, moieties chosen from the groups consisting of halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino, N- (phenylsulfonyl)amino or phenyl, wherein the phenyl is substituted or unsubstituted, such that, for example, a biphenyl results.

[0048] Examples of the term "substituted phenyl" includes a mono- or di(halo)phenyl group such as 2, 3 or 4-chlorophenyl, 2,6-difluorophenyl, 2,3-difluorophenyl, 2,6-dichlorophenyl, 2,5-dichlorophenyl, 3,4-dichlorophenyl, 2, 3 or 4-bromophenyl, 3,4-dibromophenyl, 3-chloro-4-fluorophenyl, 2, 3 or 4-fluorophenyl and the like; a mono or di(hydroxy)phenyl group such as 2, 3 or 4-hydroxyphenyl, 2,4-dihydroxyphenyl, the protected-hydroxy derivatives thereof and the like; a nitrophenyl group such as 2, 3 or 4-nitrophenyl; a cyanophenyl group, for example, 2, 3 or 4-cyanophenyl; a mono- or di(alkyl)phenyl group such as 2, 3 or 4-methylphenyl, 2,4-dimethylphenyl, 2, 3 or 4-(iso-propyl)phenyl, 2, 3 or 4-ethylphenyl, 2, 3 or 4-(n-propyl)phenyl and the like; a mono or di(alkoxyl)phenyl group, for example,

2,6-dimethoxyphenyl, 2, 3 or 4-methoxyphenyl, 2, 3 or 4-ethoxyphenyl, 2, 3 or 4-(isopropoxy)phenyl, 2, 3 or 4-(t-butoxy)phenyl, 3-ethoxy-4-methoxyphenyl and the like; 2, 3 or 4-trifluoromethylphenyl; a mono- or dicarboxyphenyl or (protected carboxy)phenyl group such as 2, 3 or 4-carboxyphenyl or 2,4-di(protected carboxy)phenyl; a mono-or di(hydroxymethyl)phenyl or (protected hydroxymethyl)phenyl such as 2, 3, or 4-(protected hydroxymethyl)phenyl or 3,4-di(hydroxymethyl)phenyl; a mono- or di(aminomethyl)phenyl or (protected aminomethyl)phenyl such as 2, 3 or 4-(aminomethyl)phenyl or 2,4-(protected aminomethyl)phenyl; or a mono- or di(N-(methylsulfonylamino))phenyl such as 2, 3 or 4-(N-(methylsulfonylamino))phenyl. Also, the term "substituted phenyl" represents disubstituted phenyl groups wherein the substituents are different, for example, 3-methyl-4-hydroxyphenyl, 3-chloro-4-hydroxyphenyl, 2-methoxy-4-bromophenyl, 4-ethyl-2-hydroxyphenyl, 3-hydroxy-4-nitrophenyl, 2-hydroxy 4-chlorophenyl, and the like.

[0049] The term "C_7 to C_{12} phenylalkyl" denotes a C_1 to C_6 alkyl group substituted at any position by a phenyl, substituted phenyl, heteroaryl or substituted heteroaryl. Examples of such a group include benzyl, 2-phenylethyl, 3-phenyl(n-propyl), 4-phenylhexyl, 3-phenyl(n-amyl), 3-phenyl(sec-butyl), and the like. Preferred C_7 to C_{12} phenylalkyl groups are the benzyl and the phenylethyl groups.

[0050] The term "C_7 to C_{12} substituted phenylalkyl" denotes a C_7 to C_{12} phenylalkyl group substituted on the C_1 to C_6 alkyl portion with one or more, and preferably one or two, groups chosen from halogen, hydroxy, protected hydroxy, oxo, protected oxo, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, guanidino, protected guanidino, heterocyclic ring, substituted heterocyclic ring, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, nitro, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-(C_1 to C_6 dialkyl)carboxamide, cyano, N-(C_1 to C_6 alkylsulfonyl)amino, thiol, C_1 to C_4 alkylthio, C_1 to C_4 alkylsulfonyl groups; and/or the phenyl group may be substituted with one or more, and preferably one or two,

substituents chosen from halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, (monosubstituted)amino, protected (monosubstituted)amino, (disubstituted)amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl) carboxamide, protected N-(C_1 to C_6 alkyl) carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino, N-(phenylsulfonyl)amino, cyclic C_2 to C_8 alkylene or a phenyl group, substituted or unsubstituted, for a resulting biphenyl group. The substituted alkyl or phenyl groups may be substituted with one or more, and preferably one or two, substituents which can be the same or different.

[0051] Examples of the term "C_7 to C_{12} substituted phenylalkyl" include groups such as 2-hydroxyphenylmethyl, 3-hydroxyphenylmethyl, 2-methoxyphenylmethyl, 3-methoxyphenylmethyl, 2,6-difluorophenylmethyl, 2,3-difluorophenylmethyl, 2,6-dichlorophenylmethyl, 2,3-dichlorophenylmethyl, 3,5-dichlorophenylmethyl, 2-hydroxyphenylethyl, 3-hydroxyphenylethyl, 2-methoxyphenylethyl, 3-methoxyphenylethyl, 2,6-difluorophenylethyl, 2,3-difluorophenylethyl, 2,6-dichlorophenylethyl, 2,3-dichlorophenylethyl, 3,5-dichlorophenylmethyl 2-phenyl-1-chloroethyl, 2-(4-methoxyphenyl)ethyl, 4-(2,6-dihydroxy phenyl)n-hexyl, 2-(5-cyano-3-methoxyphenyl)n-pentyl, 3-(2,6-dimethylphenyl)n-propyl, 4-chloro-3-aminobenzyl, 6-(4-methoxyphenyl)-3-carboxy(n-hexyl), 5-(4-aminomethylphenyl)- 3-(aminomethyl)n-pentyl, 5-phenyl-3-oxo-n-pent-1-yl, and the like.

[0052] The term "heterocycle" or "heterocyclic ring" denotes optionally substituted five-membered to eight-membered rings that have 1 to 4 heteroatoms, such as oxygen, sulfur and/or nitrogen, in particular nitrogen, either alone or in conjunction with sulfur or oxygen ring atoms. These five-membered to eight-membered rings may be saturated, fully unsaturated or partially unsaturated, with fully saturated rings being preferred. Preferred heterocyclic rings include morpholino,

piperidinyl, piperazinyl, 2-amino-imidazoyl, tetrahydrofurano, pyrrolo, tetrahydrothiophen-yl, hexamethyleneimino and heptamethyleneimino.

[0053] The term "substituted heterocycle" or "substituted heterocyclic ring" means the above-described heterocyclic ring is substituted with, for example, one or more, and preferably one or two, substituents which are the same or different which substituents can be halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, (monosubstituted)amino, protected (monosubstituted)amino, (disubstituted)amino carboxamide, protected carboxamide, N-(C_1 to C_{12} alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino, N-(phenylsulfonyl)amino, heterocycle or substituted heterocycle groups.

[0054] The term "heteroaryl" means a heterocyclic aromatic derivative which is a five-membered or six-membered ring system having from 1 to 4 heteroatoms, such as oxygen, sulfur and/or nitrogen, in particular nitrogen, either alone or in conjunction with sulfur or oxygen ring atoms. Examples of heteroaryls include pyridinyl, pyrimidinyl, and pyrazinyl, pyridazinyl, pyrrolo, furano, thiopheno, oxazolo, isoxazolo, phthalimido, thiazolo, and the like.

[0055] The term "substituted heteroaryl" means the above-described heteroaryl is substituted with, for example, one or more, and preferably one or two, substituents which are the same or different which substituents can be halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 alkoxy, C_1 to C_8 substituted alkoxy, C_1 to C_8 acyl, C_1 to C_8 substituted acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, (monosubstituted)amino, protected (monosubstituted)amino, (disubstituted)amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6

alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino or N-(phenylsulfonyl)amino groups.

[0056] The term "substituted naphthyl" specifies a naphthyl group substituted with one or more, and preferably one or two, moieties either on the same ring or on different rings chosen from the groups consisting of halogen, hydroxy, protected hydroxy, cyano, nitro, C_1 to C_8 alkyl, C_1 to C_8 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, carboxy, protected carboxy, carboxymethyl, protected carboxymethyl, hydroxymethyl, protected hydroxymethyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N, N-di(C_1 to C_6 alkyl)carboxamide, trifluoromethyl, N-((C_1 to C_6 alkyl)sulfonyl)amino or N-(phenylsulfonyl)amino.

[0057] Examples of the term "substituted naphthyl" includes a mono or di(halo)naphthyl group such as 1, 2, 3, 4, 5, 6, 7 or 8-chloronaphthyl, 2, 6-dichloronaphthyl, 2, 5-dichloronaphthyl, 3, 4-dichloronaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-bromonaphthyl, 3, 4-dibromonaphthyl, 3-chloro-4-fluoronaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-fluoronaphthyl and the like; a mono or di(hydroxy)naphthyl group such as 1, 2, 3, 4, 5, 6, 7 or 8-hydroxynaphthyl, 2, 4-dihydroxynaphthyl, the protected-hydroxy derivatives thereof and the like; a nitronaphthyl group such as 3- or 4-nitronaphthyl; a cyanonaphthyl group, for example, 1, 2, 3, 4, 5, 6, 7 or 8-cyanonaphthyl; a mono- or di(alkyl)naphthyl group such as 2, 3, 4, 5, 6, 7 or 8-methylnaphthyl, I, 2, 4-dimethylnaphthyl, I, 2, 3, 4, 5, 6, 7 or 8-(isopropyl)naphthyl, I, 2, 3, 4, 5, 6, 7 or 8-ethylnaphthyl, I, 2, 3, 4, 5, 6, 7 or 8-(n-propyl)naphthyl and the like; a mono or di(alkoxy)naphthyl group, for example, 2, 6-dimethoxynaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-methoxynaphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-ethoxynaphthyl, I, 2, 3, 4, 5, 6, 7 or 8-(isopropoxy)naphthyl, 1, 2, 3, 4, 5, 6, 7 or 8-(t-butoxy)naphthyl, 3-ethoxy-4-methoxynaphthyl and the like; 1, 2, 3, 4, 5, 6, 7 or 8-trifluoromethylnaphthyl; a mono- or dicarboxynaphthyl or (protected carboxy)naphthyl group such as 1, 2, 3, 4, 5, 6, 7 or 8-carboxynaphthyl or 2, 4-di(-protected carboxy)naphthyl; a mono-or di(hydroxymethyl)naphthyl or (protected hydroxymethyl)naphthyl such as 1, 2, 3, 4, 5, 6, 7 or 8-(protected hydroxymethyl)naphthyl or 3, 4-di(hydroxymethyl)naphthyl; a

mono- or di(amino)naphthyl or (protected amino)naphthyl such as 1, 2, 3, 4, 5, 6, 7 or 8-(amino)naphthyl or 2, 4-(protected amino)-naphthyl, a mono- or di(aminomethyl)naphthyl or (protected aminomethyl)naphthyl such as 2, 3, or 4-(aminomethyl)naphthyl or 2, 4-(protected aminomethyl)-naphthyl; or a mono- or di-(N-methylsulfonylamino) naphthyl such as 1, 2, 3, 4, 5, 6, 7 or 8-(N-methylsulfonylamino)naphthyl. Also, the term "substituted naphthyl" represents disubstituted naphthyl groups wherein the substituents are different, for example, 3-methyl-4-hydroxynaphth-1-yl, 3-chloro-4-hydroxynaphth-2-yl, 2-methoxy-4-bromonaphth-1-yl, 4-ethyl-2-hydroxynaphth-1-yl, 3-hydroxy-4-nitronaphth-2-yl, 2-hydroxy-4-chloronaphth-1-yl, 2-methoxy-7-bromonaphth-1-yl, 4-ethyl-5-hydroxynaphth-2-yl, 3-hydroxy-8-nitronaphth-2-yl, 2-hydroxy-5-chloronaphth-1-yl and the like.

[0058] As outlined above R_3 and R_4 may be taken together with nitrogen to form a heterocycle or substituted heterocycle of the following kind aziridine, azetidine, pyrrolidine, 3-methylpyrrolidine, 3-aminopyrrolidine, 3-hydroxypyrrolidine, pyrazolidine, imidazolidine, piperidine, 2-methylpiperidine, 4-carboxypiperidine, 4-(carboxymethyl)piperidine, piperazine, morpholine, azepine, and tetrahydroisoquinoline.

[0059] The term "C_1 to C_8 acyl" encompasses groups such as formyl, acetyl, propionyl, butyryl, pentanoyl, pivaloyl, hexanoyl, heptanoyl, benzoyl and the like. Preferred acyl groups are acetyl and benzoyl.

[0060] The term "C_1 to C_8 substituted acyl" denotes the acyl group substituted by one or more, and preferably one or two, halogen, hydroxy, protected hydroxy, oxo, protected oxo, cyclohexyl, naphthyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, guanidino, heterocyclic ring, substituted heterocyclic ring, imidazolyl, indolyl, pyrrolidinyl, C_1 to C_8 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, nitro, C_1 to C_8 alkyl ester, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N,N-di(C_1 to C_6 alkyl)carboxamide, cyano, methylsulfonylamino, thiol, C_1 to C_4 alkylthio or C_1 to C_4 alkylsulfonyl groups. The

substituted acyl groups may be substituted once or more, and preferably once or twice, with the same or with different substituents.

[0061] Examples of C_1 to C_8 substituted acyl groups include 4-phenylbutyroyl, 3-phenylbutyroyl, 3-phenylpropanoyl, 2- cyclohexanylacetyl, cyclohexanecarbonyl, 2-furanoyl and 3-dimethylaminobenzoyl and the like.

[0062] The term "C_1 to C_8 alkoxy" as used herein denotes groups such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, t-butoxy and like groups. A preferred alkoxy is methoxy. The term "C_1 to C_8 substituted alkoxy" means the alkyl portion of the alkoxy can be substituted in the same manner as in relation to C_1 to C_8 substituted alkyl.

[0063] The term "C_1 to C_8 substituted aminoacyl" denotes the acyl group substituted by one or more, and preferably one or two, halogen, hydroxy, protected hydroxy, oxo, protected oxo, cyclohexyl, naphthyl, amino, protected amino, monosubstituted amino, protected monosubstituted amino, disubstituted amino, guanidino, heterocyclic ring, substituted heterocyclic ring, imidazolyl, indolyl, pyrrolidinyl, C_1 to C_8 alkoxy, C_1 to C_8 acyl, C_1 to C_8 acyloxy, nitro, C_1 to C_8 alkyl ester, carboxy, protected carboxy, carbamoyl, carboxamide, protected carboxamide, N-(C_1 to C_6 alkyl)carboxamide, protected N-(C_1 to C_6 alkyl)carboxamide, N,N-di(C_1 to C_6 alkyl)carboxamide, cyano, methylsulfonylamino, thiol, C_1 to C_6 alkylthio or C_1 to C_6 alkylsulfonyl groups. The substituted acyl groups may be substituted once or more, and preferably once or twice, with the same or with different substituents.

[0064] This invention provides a pharmaceutical composition comprising an effective amount of a compound according to the invention. Such compositions can be administered by various routes, for example oral, rectal, subcutaneous, intramuscular, intravenous or intracerebral. The preferred route of administration would be oral at daily doses of the compound for adult human treatment of about 0.01-5000 mg, preferably 1-1500 mg per day. The appropriate dose may be administered in a single dose or as divided doses presented at appropriate intervals for example as two, three four or more subdoses per day.

[0065] For preparing pharmaceutical compositions containing compounds of the invention, inert, pharmaceutically acceptable carriers are used. The pharmaceutical carrier can be either solid or liquid. Solid form preparations include, for example, powders, tablets, dispersible granules, capsules, cachets, and suppositories.

[0066] In particular, the invention relates to compounds as described above wherein the compounds are capable of binding the NR1H4 receptor protein or a portion thereof according to SEQ ID NOS 1-4 shown in Figs. 4A-4D, respectively, or a mammalian homologue thereof. The claimed compound can bind to the NR1H4 receptor protein or a portion thereof in a mixture comprising 10-200 ng of NR1H4 receptor protein or a portion thereof, preferably the ligand binding domain, 20 mM Tris /HCl at pH 7.9; 60 mM KCl; 5 mM $MgCl_2$; 160ng/µl BSA in a total volume of preferably about 25 µl.

[0067] A mammalian receptor protein homologue of the protein according to SEQ ID NO. 1 shown in Fig. 4A as used herein is a protein that performs substantially the same function as NR1H4 does in humans and shares at least 40% sequence identity at the amino acid level, preferably 50% sequence identity at the amino acid level more preferably 65% sequence identity at the amino acid level, even more preferably 75% sequence identity at the amino acid level and most preferably over 85% sequence identity at the amino acid level.

[0068] The invention in particular concerns a method for prevention or treatment of a NR1H4 receptor protein- or NR1H4 receptor protein homologue-mediated disease or condition in a mammal comprising administration of a therapeutically effective amount of a compound according to the invention wherein the prevention or treatment is directly or indirectly accomplished through the binding of a compound according to the invention to the NR1H4 receptor protein or to the NR1H4 receptor protein homologue.

[0069] The term mediated herein means that the physiological pathway in which the NR1H4 receptor protein acts is either directly or indirectly involved in the disease or condition to be treated or prevented. In the case where it is indirectly involved it could be that, e.g. modulating the activity of NR1H4 by a compound according to the invention influences a parameter which has a beneficial effect on a disease or a condition. One such example is that modulation of NR1H4 activity leads to decreased levels of serum cholesterol or certain lipoproteins, which, in turn, have a beneficial effect on the prevention and treatment of artherosclerosis. Herein a condition is a physiological or phenotypic state which is desirably altered. One such example would be obesity which is not necessarily medically harmful but nonetheless a non desirable phenotypic condition. In a preferred embodiment of the invention the method for prevention or treatment of a NR1H4 receptor protein mediated disease or condition is applied to a human. This may be male or female.

[0070] Pharmaceutical compositions generally are administered in an amount effective for treatment or prophylaxis of a specific condition or conditions. Initial dosing in a human is accompanied by clinical monitoring of symptoms, such symptoms being determined for the selected condition. In general, the compositions are administered in an amount of active agent of at least about 100 µg/kg body weight. In most cases they will be administered in one or more doses in an amount not in excess of about 20 mg/kg body weight per day. Preferably, in most cases, the dose is from about 100 µg/kg to about 5 mg/kg body weight, daily.

[0071] For administration particularly to mammals, and particularly humans, it is expected that the daily dosage level of active agent will be 0.1 mg/kg to 10 mg/kg and typically around 1 mg/kg.

[0072] By "therapeutically effective amount" is meant a symptom- alleviating or symptom -reducing amount, a cholesterol-reducing amount, a fatty acid absorption blocking amount, a protein and/or carbohydrate digestion-blocking amount and/or a de novo cholesterol biosynthesis-blocking amount of a compound according to the invention. The term "blocking" as used herein means either total blockage or partial blockage.

[0073] FXR is proposed to be a bile acid sensor. As a result, it modulates both the synthetic output of bile acids in the liver and their recycling in the intestine, by regulating bile acid binding proteins. In one embodiment of the invention the invention concerns a method for regulating bile transport in a mammal, in a preferred embodiment a human, which comprises activating the NR1H4 receptor with a therapeutically effective amount of a compound according to the invention.

[0074] Likewise the invention concerns a method of treating in mammal a disease which is affected by cholesterol, triglyceride, or bile acid levels comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to the invention.

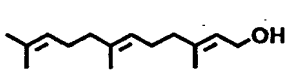
[0075] Accordingly, the compounds according to the invention may also be used as a method of prevention or treatment of mammalian atherosclerosis, gallstone disease, lipid disorders, obesity or cardiovascular disorders such as coronary heart disease or stroke.

[0076] The invention further concerns a method of blocking fatty acid absorption in the intestine of a mammal comprising administering to the mammal a therapeutically effective amount of a compound according to the invention. The invention may also be used to treat obesity in a mammal, particularly in humans.

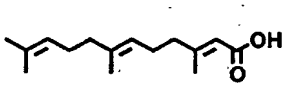
[0077] FXR alpha is a prototypical type 2 nuclear receptor which activates genes upon binding to the promoter region of target genes in a heterodimeric fashion with RXR. The relevant physiological ligands of NR1H4 are bile acids. The present compounds according to the invention have been demonstrated to have high binding efficacy binding coefficients measured as IC50 in the range 400 nM to 1000 nM as well as agonistic and/or antagonistic properties. Consequently they may be applied to regulate genes that participate in bile acid homeostasis as well as other downstream regulated genes. Examples of such genes are, but are not limited to, genes encoding proteins or factors involved directly or indirectly in lipid absorption, cholesterol biosynthesis, cholesterol transport or binding, bile acid transport or

binding, proteolysis, amino acid metabolism, glucose biosynthesis, protein translation, electron transport, and hepatic fatty acid metabolism. FXR often functions in vivo as a heterodimer with the RXR. Published FXR agonists such as the Glaxo SmithKline compound "GW 4064" are known to influence the regulation of various liver genes. Examples of known agonists are showin in Table 1 below.

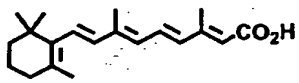
TABLE 1



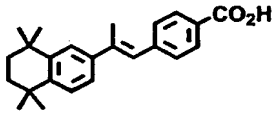
Farnesol



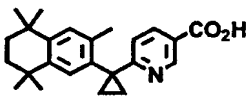
Farnesoic acid



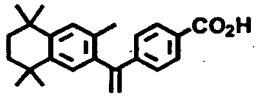
All *trans*-Retinoic acid



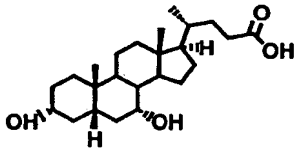
TTNPB



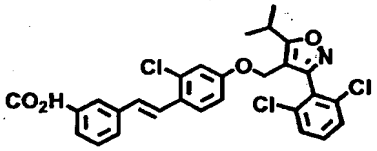
LG 268



LG 69



Carboxysteroids
CDCA



Ethers
GW 4064

[0078] Genes found to be regulated by GW 4064 are genes down-regulated in the liver, genes up-regulated in the liver and genes having altered expression in the intestine.

[0079] Genes down-regulated in the liver include apolipoprotein B; plasma proteinase inhibitor alpha-1-inhibitor III group 3(m22360); L-glucono-gamma-lactone oxidase (d12754); peroxisomal enoyl-CoA:hydrotase-3-hydroxyacyl-CoA bifunctional enzyme (k03249); liver fatty acid binding protein (L-FABP, m13501), CYP4A2 (m57719), CYP3A23 (x96721) and CYP3A1 (x64401); Cholesterol-7-alpha-hydroxylase, CYP7A1 (RefSeq NM000780, XM 005022, XM 044651, and XM 044652); and sodium-taurocholate cotransport protein, ntcp (RefSeq NM003049, XM007466).

[0080] Genes that up-regulate in the liver include small heterodimer partner homolog (d86580); bile salt export pump, bsep (RefSeq NM 003742, XM 003644,

and XM 033122); phospholipid transfer protein, PLTP (RefSeq NM 006227, XM 009490, XM 029929, and XM 029930); arnithine palmitoyltransferase II, CPTII (RefSeq NM 000098, XM 001758, XM 038866, and XM 038867); phenylethanolamine-N-methyltransferase, PNMT (RefSeq NM 002686, XM 008597, andXM 049837); insulin-induced growth-response protein CL-6 (I13619); elongation factor 2, EF-2 (y07504); mouse cornichon; protein kinase C receptor (u03390); mitochondrial cytochrome c oxidase (m27315); cystathione gamma-lyase (x53460, d17370); cytosolic phosphoenolypyruvate carboxykinase (k03243); histidase (m58308); S-adenosylmethionine synthetase (x60822); lanosterol 14-alpha-demethylase (u17697); G protein-coupled purinoceptor P2U (146865) and hepatic squalene synthetase (m95591).

[0081] Genes having altered expression in the intestine include lipase (x61925); pancreatic lipase (d88534); colipase (m58370); pancreatic phospholipase A-2 (d00036); pancreatic amylase (m24962); carboxypeptidase A1 (m23986); carboxypeptidase A2 (m23721): carboxypeptidase B (m23959): pancreatic trypsin I (j00778): pancreatic cationic trypsinogen (m16624); pancreatic trypsinogen II (v01274); elastase I (v01234, I00112); elastase II (I00118, I00124); I-BABP (I22788); intestinal fatty acid binding protein (FABP, k01180); hepatic squalenesynthetase (m95591); protein kinase C receptor (u003390): longation factor 2, EF-2 (y07504) and small heterodimer partner homolog (d86580).

[0082] Thus, the invention also concerns a method of modulating a gene whose expression is regulated by the NR1H4 receptor in a mammal comprising administration of a therapeutically effective amount of a compound according to the invention to the mammal.

[0083] It is known that the orphan receptor FXR can bind the response element of the SHP gene as a heterodimer with RXR (9-cis retinoic acid receptor) and the SHP-protein, in turn, prevents efficient transcription from the cyp7a1 promoter (Lu et al., Mol Cell, 6(3):505-17; Goodwin et al. Mol Cell, 6(3), 717-26, 2000). Another gene that is repressed via SHP upon FXR activation is the sodium/bile acid cotransporter gene, NTCP, a membrane transport protein which is

required for the import of conjugated bile acids in the hepatocyte (Denson et al., Gastroenterology;121(1):218-20, 2001). The gene for the bile salt export pump, a membrane transporter responsible for the secretion of bile acids into the gall is directly activated by FXR (Ananthanarayanan et al., J Biol Chem, 3;276(31):28857-28865, 2001). Consequently, the invention likewise concerns a method for lowering the expression of cholesterol 7-alpha-hydroxylase and NTCP and increasing expression of BSEP in parallel by use of the compounds according to the invention. In one embodiment the invention concerns a method for enhancing the expression of the Intestinal Bile Acid Binding Protein (I-BABP) (Grober et al., J Biol Chem, 15;274(42):29749-54, 1999 and/or the activity of the canicular bile salt excretion pump.

[0084] The compounds according to the invention may be used as medicaments, in particular for the prevention or treatment of a NR1H4 receptor protein- or NR1H4 receptor protein homologue- mediated disease or condition in a mammal wherein the prevention or treatment is directly or indirectly accomplished through the binding of the compound according to the invention to the NR1H4 receptor protein or NR1H4 receptor protein homologue. These pharmaceutical compositions contain 0.1% to 99.5% of the compound according to the invention, more particularly 0.5% to 90% of the compound according to the invention in combination with a pharmaceutically acceptable carrier.

[0085] The invention also concerns the use of a compound or combination of compounds according to the invention for the prevention or treatment of a NR1H4 receptor protein-mediated disease or condition wherein the mammal described above is a human. The medicament may be used for regulating the bile transport system in a mammal, preferentially a human, by activating the NR1H4 receptor, for regulating levels of cholesterol, triglyceride, and/or bile acid. For example, the medicament may be used for the treatment of atherosclerosis, gallstone disease, lipid disorders, obesity or a cardiovascular disorder.

[0086] The invention further concerns the use of a compound or combination of compounds according to the invention for blocking in a mammal, preferentially a

human, fatty acid absorption in the intestine. Further the compounds of the invention may be used for treating obesity in humans and for modulating a gene whose expression is regulated by the NR1H4 receptor (see details above and figures). The invention also further concerns the use of a compound or combination of compounds as antitumor medicaments. The antitumor effects of such medicaments could be exerted by selective inhibition of cell proliferation and induction of apotptosis of tumor cells in a way similar to described activities for certain bisphosphonates (Alberts DS, et al., Clin Cancer Res 2001 May;7(5):1246-50).

[0087] The following examples illustrate a specific embodiment of the invention, but they are not to be considered as limiting the invention in any manner.

EXAMPLE 1

[0088] For *in vitro* screening for compounds which influence FXR binding to coactivators, a fragment of the open reading frame of human FXR alpha (NR1H4 - (Acc. No:AF384555)) encoding aminoacids 187-472 was amplified by standard RT PCR procedures (see SEQ ID NOS. 1 and 2 in Figs. 4A and 4B, respectively). The starting material was total RNA derived from human liver. The resulting cDNA obtained after reverse transcription was subsequently cloned using the Gateway™ recombination technology (Invitrogen, USA) into the expression plasmid pDest15 (Invitrogen, USA). This construct was used to express a recombinant GST-FXR fusion protein in E.coli (BL21 strain). A pDEST 17 derivative clone harboring an additional sequence encoding amino acids 548-878 of human TIF2 (Acc. No: XM_011633 RefSeq) was constructed using Gateway™ recombination technology (Invitrogen, USA) in order to obtain a construct which was used to express recombinant His-tagged TIF2 fragment in *E. coli*. For *E. coli* expression of both constructs, plasmid DNA was transformed into chemically competent E. coli BL21 (Invitrogen, USA) and cells were grown to an OD600 of 0.4-0.7 before expression was induced by addition of 0.5 mM IPTG according instructions of the manufacturer (Invitrogen). After induction for 8 hours at 30°C, the cells were harvested by centrifugation for 10 minutes at 5000 x g. Fusion proteins were affinity purified using Glutathion sepharose (Pharmacia) or Ni-NTA Agarose (QIAGEN) according to the

instructions of the respective manufacturer. Recombinant proteins were dialyzed against 20 mM Tris/HCL pH 7.9; 60 mM KCl; 5 mM $MgCl_2$; 1 mM DTT, 0.2 mM PMSF; 10% glycerol. The TIF2 fragment was subsequently biotinylated by addition of 40-120 µl of a biotinamidocaproate N-hydroxysuccinimide-ester (Sigma) solution (20 mg/ml in DMSO). Overhead rotating samples were incubated for 2 hours at room temperature. Unincorporated label was then separated using G25 Gel filtration chromatography (Pharmacia Biotech, Sweden). Protein containing fractions from the column were pooled and tested for activity in the assay as described below.

[0089] For screening of the compound libraries as provided for by the methods described in Examples 2, 3 and 4 below for substances which influence the FXR/Tif 2 interaction, Perkin Elmer LANCE technology was used. This technoligy relies on the binding dependent energy transfer from a donor to an acceptor fluorophore attached to the binding partners of interest. For ease of handling and reduction of background from compound fluorescence, LANCE technology makes use of generic fluorophore labels and time resoved detection. For a detailed description of this technoligy, see Hemmilä I, Blomberg K and Hurskainen P, Time-resolved resonance energy transfer (TR-FRET) principle in LANCE, Abstract of Papers Presented at the 3 rd Annual Conference of the Society for Biomolecular Screening, Sep., California (1997).

[0090] For screening, 20-200 ng of biotinylated Tif 2 fragment and 10-200 ng of GST-FXR fragment were combined with 0.5-2 nM LANCE Eu-(W1024) labelled anti-GST antibody (Perkin Elmer) and 0.5-2 µg of highly fluorescent APC-labeled streptavidin (Perkin Elmer) in the presence of 50 µM of individual compounds to be screened in a total volume of 25 µl of 20 mM Tris /HCl pH 7.9; 60 mM KCl; 5 mM MgCl2; and 160 ng/µl BSA. DMSO content of the samples was kept below 4%. The samples were incubated for a minimum of 60 minutes in the dark at room temperature in FIA-Plates black 384well med. binding (Greiner).

[0091] The LANCE signal was detected by a Perkin Elmer VICTOR2V™ Multilabel Counter applying the detection parameters listed in Table 2 below.

TABLE 2

Number of repeats 1
plate: GREINER FIA-Plate black 384 well med. binding
Measurement height ..... 3.50 mm
Label technology ..... TR-F Lance
Emission filter name D615
Emission filter slot A1
Emission aperture ..... Normal
Excitation filter D340
Delay ..... 50 µs
Window time ..... 400 µs
Cycle ..... 1000 µs
Light integrator capacitors 1
Light integrator ref. level 95
Flash energy area ..... High
Flash energy level 223
Flash absorbance measurement ..... No
Beam ..... Normal
Label technology ..... TR-F Lance
Emission filter name D665
Emission filter slot A8
Emission aperture ..... Normal
Excitation filter D340
Delay ..... 50 µs
Window time ..... 400 µs
Cycle ..... 1000 µs
Light integrator capacitors 1
Light integrator ref. level 95
Flash energy area ..... High
Flash energy level 223
Flash absorbance measurement ..... No
Beam ..... Normal

[0092] The results were visualized by plotting the ratio between the emitted light at 665 nm and at 615 nm. For every batch of recombinant proteins amount of proteins and labeling reagents giving the most sensitive detection of hits were determined individually by analysis of dose response curves for chenodeoxycholic acid.

[0093] The methods of preparing 2-aminothiazole derivative compounds and combinatorial libraries are set forth in Examples 2, 3 and 4 below.

EXAMPLE 2

[0094] The following steps describe the experimental procedure for the preparation of the compounds according to the invention utilizing ArgoGel-MB-CHO resin. The synthesis scheme is shown in Figs. 1A and 1B.

Step 1. Synthesis of Chalcone (compound 1)

[0095] To a solution of acetophenone (0.05 mol) and aldehyde (0.05 mol) in 150 ml of MeOH was added 2.5 eq of NaOH as pellets. The reaction bottle was placed on a shaker (slightly exothermic reaction) for 24-48 hrs. Then the reaction container was placed in an acetone-ice bath (-5 to 0 °C) and the reaction mixture was quenched with 10.3 ml of 37 % aqueous HCl (2.5 eq). Solvent was removed *in vacuo* and the residue was partitioned between EtOAc and water. The aqueous layer was discarded and the organic layer was washed with water, brine and dried over Na_2SO_4. Solvent was removed *in vacuo* and the crude chalcone (compound 1) was recrystallized from methanol (MeOH) or a mixture of hexane and ethyl acetate (Hex:EtOAc) 3:1 or purified by silica gel chromatography using a mixture of Hex:EtOAc = 5:1 as an eluent.

Step 2. Preparation of 1,3-Diphenylpropan-1-one (compound 2)

[0096] Chalcone (compound 1) (37.6 mmol) was dissolved in 250 ml of toluene (in some cases 25 ml of MeOH was added to insure a complete dissolution) and 400 mg of 10% Pd/C (0.01 eq) was added to the mixture. The compound was

hydrogenated at 45 psi in a Parr apparatus for 40 min to 3 hrs to form 1,3-diphenylpropan-1-one (compound 2), during which time the reaction mixture was periodically checked for the starting material by TLC (Hex:EtOAc=1:1) to prevent over-reduction. Upon completion of reduction the reaction mixture was filtered through a short pad (2-3 inch) of silica gel and solvent was removed *in vacuo* to afford a crude product, which was either sufficiently pure to use in the next step or was purified by recrystallization from MeOH or Hex:EtOAc=3:1 or, in the case where over-reduction has occurred, by silica gel column chromatography using Hex:EtOAc=5:1 as an eluent.

Step 3. Preparation of 2-Bromo-1,3-Diphenylpropan-1-one (compound 3)

[0097] To a solution of 1,3-diphenylpropan-1-one (compound 2) (7.0 mmol) in 25 ml of anhydrous dioxane, a solution of bromine (7.0 mmol) in 10 ml of dioxane was slowly added dropwise over 30 min. The reaction mixture was left to stir at room temperature for 24 hrs to form 2-bromo-1,3-diphenylpropan-1-one (compound 3). Solvent was removed *in vacuo*. The 2-bromo-1,3-diphenylpropan-1-one product formed was determined by ^{1}H NMR to be sufficiently pure to be used in the next step.

Step 4. Reductive Amination of Argogel-MB-CHO Resin (4)
with Primary Amines to form Aminated Argogel-MB-HCO resin (5)

[0098] Argogel-MB-CHO resin (Argonaut Technologies Inc.) (100 mg each tea-bag [Houghten, U.S. Patent No. 4,631,211], 0.41 mmol/g substitution) (compound 4) was swollen in 1% acetic acid (AcOH) in DMF (by volume). The amine (10 eq.) was added and the bottle(s) placed on a shaker for 30 min. Solid $NaBH_3CN$ (20 eq) was added and the reaction bottle(s) placed on a shaker at room temperature for 18 hrs to form resin aminated Argogel-MB-HCO resin (5). The resin was washed as follows: DMF (4x), MeOH (4x), CH_2Cl_2 (2x) and then allowed to air dry. For the amines that were hydrochloride salts, 1 eq of Et_3N was added.

Step 5. Preparation of a resin-bound thiourea (compound 7)

[0099] A 0.2 M solution of Fmoc-NCS (5 eq) in anhydrous CH_2Cl_2 was added to a bottle containing the aminated Argogel-MB-HCO resin (resin compound 5). The bottle was placed on a shaker for one hour to form resin compound 6. The resin was washed with CH_2Cl_2 (3x) and DMF (3x) and subsequently reacted with 20 % piperidine in DMF (5 eq) for one hour to produce a resin-bound thiourea (resin compound 7). The resin was then washed with DMF (3x) and MeOH (3x) and used directly in the next step.

Step 6. Preparation of the resin-bound 2-aminothiazole (compound 8)

[00100] The resin was placed a reaction bottle and the resin was swollen in MeOH and $NaHCO_3$ (10 eq) was added to the solution. The reaction bottle was placed on a shaker for 10 min, then a 0.2 M solution of 2-bromo-1,3-diphenylpropan-1-one (15 eq) (compound 3) in MeOH was added to the mixture and the bottle was placed on a shaker for 24 hrs to form resin-bound 2-aminothiazole. Then the solution mixture was decanted and the resin was washed with MeOH (4x), CH_3CN/H_2O=1/1 (3x), CH_3CN (2x) and was taken directly to the next step.

Step 7. Hydrolysis of the ester 8 to the acid (compound 9)

[0101] Tea-bags were placed in a bottle containing 0.5 M solution of $LiOH^*H_2O$ (100 eq) in a mixture CH_3CN/H_2O=3/1. For the purpose of this example, R_3 in compound 8 in Fig. 1B is $-(CH_2-CH_2)_n-CH_2-CO_2CH_3$ where n is an integer from 0 to 8, preferably 1 to 6 and more preferably 1 to 4. The bottle was placed on a shaker for 24 hrs. Then bags were washed with CH_3CN/H_2O=3/1 (3x), H_2O (2x), then briefly treated with 1N HCl (1 min, 1x), then washed with water (3x), CH_3CN/H_2O=3/1 (3x), CH_3CN (2x), CH_2Cl_2 (2x) and air-dried to form acid compound 9.

Step 8. Cleavage of 2-aminothiazole (compound 10) from resin

[0102] Tea-bags (100 mg of resin each) were placed in vials and 3 ml of 95 % aqueous TFA were added to each vial. The vials were tightly capped and placed in an oven-shaker at 50 °C for 3 hrs. The eluate was collected, combined with one

subsequent TFA wash and the solvent was removed with a Genevac. The crude material was analyzed by LCMS (Thermo Finnigan LCQ-classic) and if necessary purified by HPLC. The product from was a 2-aminothiazole (compound 10).

EXAMPLE 3

[0103] Experimental procedure for the preparation of the compounds according to the invention utilizing Wang resin. The synthesis scheme is shown in Fig. 2.

Step 1. Immobilization of a carboxylic acid (compound 11) on Bromo-Wang resin

[0104] Bromo-Wang resin (100 mg each tea-bag, 1.00 mmol/g substition) was swollen in anhydrous DMF. A carboxylic acid (compound 11) (5 eq.) was added, followed by potassium iodide (5 eq) and cesium carbonate (5 eq), and the bottle(s) placed in an oven-shaker at 80 °C for 3 days. Then the reaction mixture was decanted and the resin was washed as follows: DMF (4x), MeOH (4x), CH_2Cl_2 (2x) and then allowed to air dry to form resin compound 12.

Step 2. Preparation of the resin-bound thioureas (compound 15)

[0105] Resin compound 12 was placed in a bottle and the resin compound was swollen in 20 % piperidine in DMF (5 eq). Tea-bags were placed in the bottle and the bottle was placed on a shaker at room temperature for one hour. Then the resin was washed with DMF (3x), MeOH (3x), CH_2Cl_2 (3x) to form resin compound 13. Then a 0.1 M solution of Fmoc-NCS (5 eq) in anhydrous CH_2Cl_2 was applied to resin compound 13 in the bottle and the bottle was placed on a shaker for one hour. The resin was then washed with CH_2Cl_2 (3x) and DMF (3x) and subsequently reacted again with 20 % piperidine in DMF (5 eq) for one hour to produce the resin-bound thiourea (compound 15). The resin was then washed with DMF (3x) and MeOH (3x) and used directly in the next step.

Step 3. Preparation of a Resin-Bound 2-Aminothiazole (compound 16)

[0106] The resin-bound thiourea was placed in a reaction bottle and the resin was swollen in MeOH and $NaHCO_3$ (5 eq) was added to the solution. The reaction

bottle was placed on a shaker for 10 min, then a 0.1 M solution of 2-bromo-1,3-diphenylpropan-1-one (compound 3) (5 eq) in MeOH was added to the mixture and the bottle was placed on a shaker for 2 days. Then the solution mixture was decanted and the resin was washed with MeOH (4x), CH_3CN/H_2O=1/1 (3x), CH_3CN (2x), CH_2Cl_2 (2x) and air-dried to form resin-bound 2-aminothiazole (compound 16).

Step 4. Cleavage of 2-aminothiazole to form products 10 from resin.

[0107] Tea-bags (100 mg of resin each) were placed in vials and 3 ml of 50 % TFA in CH_2Cl_2 were added to each vial. The vials were tightly capped and placed on a shaker at room temperature for 3 hrs. The eluate was collected and combined with one subsequent TFA wash. Then the solvent was removed with a Genevac. The crude material was analyzed by LCMS (Thermo Finnigan LCQ-classic), and if necessary purified by HPLC-MS, to form product 10.

EXAMPLE 4

[0108] Experimental procedure for the preparation of the compounds according to the invention utilizing solution-phase chemistry is shown in Fig. 3.

Step 1. Preparation of a Fmoc-Protected Thiourea (compound 18)

[0109] To a solution of an HCl salt of amine compound 17 (25 mmol) in 200 ml of anhydrous CH_2Cl_2, Et_3N (25 mmol) was added which was followed by Fmoc-NCS (25 mmol). The reaction mixture was stirred at room temperature for 2 hrs. Then solvent was removed *in vacuo* to give a crude bright-yellow solid Fmoc-protected thiorurea product (compound 18) in a quatitative yield.

Step 2. Fmoc-deprotection of Thiourea Compound 18

[0110] The crude product (compound 18) from the previous step (25 mmol) was dissolved in 20 ml of DMF and 1 ml of piperidine was added. Evolution of gas (CO_2) was observed within 5 min and the reaction was slightly exothermic. The

reaction mixture was left to stir at room temperature overnight. Then 300 ml of H_2O were added and the mixture was extracted extensively with EtOAC (150 ml, 5x). The combined extracts were dried over Na_2SO_4, solvent was removed *in vacuo* to give a yellow solid Fmoc-deprotected thiourea compound 19, to which 50 ml of CH_2Cl_2 were added and the suspension was filtered. The precipitate, compound 19, was collected on a filter, washed with a small amount of CH_2Cl_2 and dried *in vacuo*.

Step 3. Formation of a 2-aminothiazole (compound 20)

[0111] To a solution comprising 2-bromo-1,3-diphenylpropan-1-one (compound 3) (1.0 mmol) and Fmoc-deprotected thiourea (compound19) (1.0 mmol) in 15 ml of MeOH, $NaHCO_3$ (1.0 mmol) was added and the reaction mixture was heated at 50°C for 24 hours. Then solvent was removed *in vacuo*, and the residue was partitioned between EtOAc and water to form 2-aminothiazole (compound 20) which was washed with brine, dried over Na_2SO_4. Solvent was removed *in vacuo* and the residue was purified by silica gel chromatography using Hex:EtOAc = 3:1 as eluent.

Step 4. Hydrolysis of Ester Compound 20 to Acid Product 10

[0112] To a solution of the 2-aminothiazole compound 20 (2.0 mmol) in 9 ml of CH_3CN, a solution of $LiOH^*H_2O$ in 3 ml of H_2O was added. The mixture was stirred at room temperature for 24 hours, then it was acidified to pH 3 by 1 N HCl and extracted with EtOAc (3x). The extracted product was washed with brine, dried over Na_2SO_4 and solvent was removed *in vacuo* to give the product 10 as a light-yellow solid, which was purified by HPLC.

[0113] Tables 3, 4, 5 and 6 illustrate the preferred compounds according to the invention that can mediate transactivation of FXR mediated transcription in a HEK293 reporter cell line. The data summarized in the Tables below which shows the internal molecular name used by Applicant (MOLNAME) as well as the corresponding structures of preferred compounds accoring to the invention. The

Table provides the EC_{50} values (EC50 AGV) as established as well as their respective average efficacy (% activity relative to CDCA control agonist).

TABLE 3

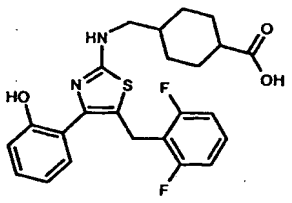
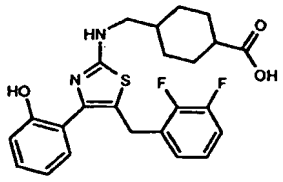
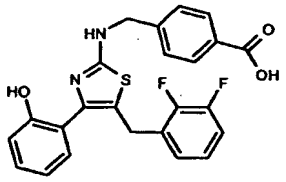
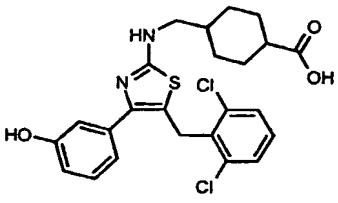
MOLNAME	MOLECULAR STRUCTURE	EC50 AVG	EFFIC AVG	EXPECTED MASS	FOUND MASS
LN0000006316		0.20	110	458.53	459.28
LN0000006322		1.45	115	459.53	459.29
LN0000006323		0.44	86	452.48	453.22
LN0000006365		0.36	92	491.44	492.21

TABLE 4

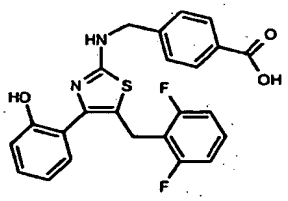
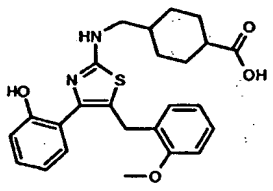
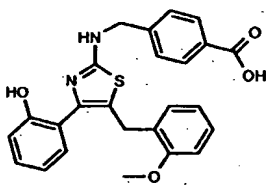
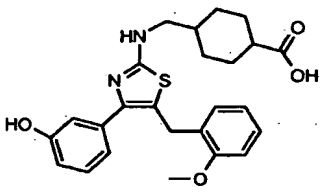
MOLNAME	MOLECULE STRUCTURE	EC50 AVG	EFFIC AVG	EXPECTED MASS	FOUND MASS
LN0000006317		1.45	115	452.48	453.22
LN0000006328		4.50	116	452.58	459.26
LN0000006329		0.73	105	446.53	447.23
LN0000006339		2.32	86	458.43	459.28

TABLE 5

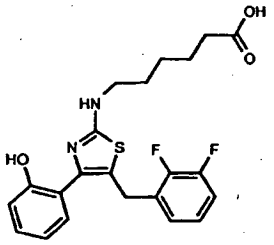
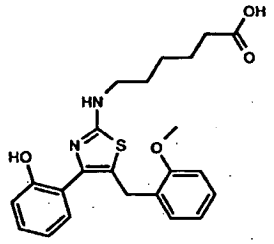
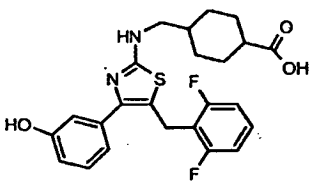
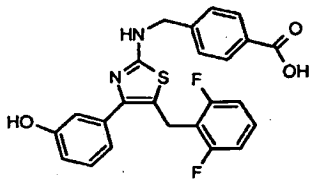
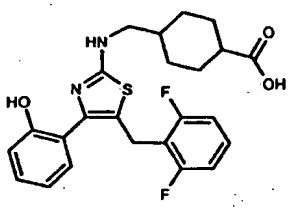
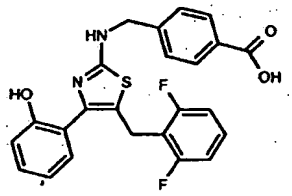
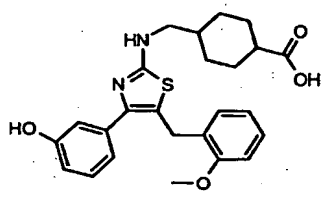
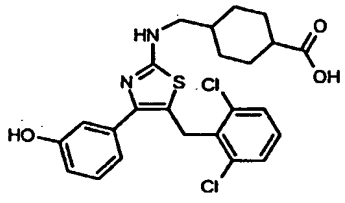
MOLNAME	MOLECULAR STRUCTURE	EC50 AVG	EFFIC AVG	EXPECTED MASS	FOUND MASS
LN0000006346		5.12	63	432.49	433.25
LN0000006347		3.10	66	426.54	427.26
LN0000006348		2.39	109	458.53	459.29
LN0000006349		4.05	66	452.48	453.22

TABLE 6

MOLNAME	MOLECULAR STRUCTURE	ED50 AVG	EFFIC AVG
LN0000006316		12	309
LN0000006317		15	223
LN0000006339		20	323
LN0000006365		15	340

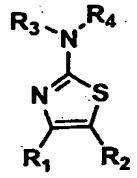
[0114] Stable HEK293FXR reporter cell lines were generated by stably transfecting with the pTRexDest30 (Invitrogen) derivatives pTRexDest30-hFXR, pTRexDest30-hRXR and the pGL2promoter (Promega) derivative pGL2promoter-FXRRE. The full length human FXR (accession U68233) and the full length human RXRα (accession P19793) were cloned into the pTRexDest30 applying the manufacturer protocols for the Gateway™ system (Invitrogen).

{0115] The FXR response elements were cloned (upper case and undrelined). 5' - cccaGGGTGAaTAACCTcggggctctgtccctccaatcccaGGGTGAaTAACCTcggg 3' (SEQ ID NO. 5) was created from the human IBAB-P promoter (Grober et al 1999, JBC 274, pp. 29749-29754). A stable clone was selected and seeded at a density of $1x10^4$ cells per well in 96 well plates. Luciferase reporter activity was measured in triplicates from extracts of cells after incubating cells in culture medium (DMEM [Gibco-BRL] + 10% FCS [PAA laboratories]) for 16 hours (5% CO_2, 37°C) containing 0.5% DMSO (control) or 0.5% DMSO with increasing concentrations of compounds. Examples of such dose response assays in the HEK293-FXR cell line are shown in Fig. 5A for LN6348, in Fig 5B for LN6316, in Fig.5C for LN6365 and in Fig. 5D for LN6322. One can derive EC50 values for the potency in the cellular reporter assay and as an example LN6348 can be determined in such an experiment with an EC50 of 1.3 µM and an relative efficacy compared to GW4064 of 109%.

[0116] While the salient features have been illustrated and described with respect to particular embodiments, it should be readily apparent that modifications can be made within the spirit and scope of the invention, and it is, therefore, not desired to limit the invention to the exact details shown and described.

What is claimed is:

1. A compound including resolved diastereoisomers and enantiomers, and tautomers, pharmaceutical acceptable salts or solvates thereof, having the following formula (I):



(I)

wherein:

R_1 is C_1 to C_7 alkyl, C_1 to C_7 substituted alkyl, phenyl, substituted phenyl, alkylphenyl, substituted alkylphenyl, C_2 to C_7 heterocyclic ring, C_2 to C_7 substituted heterocyclic ring, C_5 to C_6 heteroaryl, and C_5 to C_6 substituted heteroaryl;

R_2 is C_1 to C_7 alkyl, C_1 to C_7 substituted alkyl, phenyl, substituted phenyl, alkylphenyl, substituted alkylphenyl, C_2 to C_7 heterocyclic ring, C_2 to C_7 substituted heterocyclic ring, C_5 to C_6 heteroaryl, and C_5 to C_6 substituted heteroaryl.

R_3 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl, C_7 to C_{12} substituted alkylphenyl, naphthyl, substituted naphthyl, C_1 to C_8 alkanesulfonyl, benzenesulfonyl, substituted benzenesulfonyl, and C_1 to C_8 substituted acyl;

R_4 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl, C_7 to C_{12} substituted alkylphenyl, naphthyl, substituted naphthyl, C_1 to C_8 alkanesulfonyl, benzenesulfonyl, substituted benzenesulfonyl, C_1 to C_8 substituted acyl;

where R_3 and R_4 may be taken together with nitrogen to form a heterocycle or substituted heterocycle.

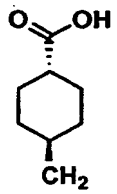
2. The compound of claim 1, wherein:

R_1 is C_1 to C_7 alkyl, C_1 to C_7 substituted alkyl, phenyl, substituted phenyl, alkylphenyl, substituted alkylphenyl, C_2 to C_7 heterocyclic ring, C_2 to C_7 substituted heterocyclic ring, C_5 to C_6 heteroaryl, or C_5 to C_6 substituted heteroaryl;

R_2 is C_1 to C_7 alkyl, C_1 to C_7 substituted alkyl, phenyl, substituted phenyl, alkylphenyl, substituted alkylphenyl, C_2 to C_7 heterocyclic ring, C_2 to C_7 substituted heterocyclic ring, C_5 to C_6 heteroaryl, or C_5 to C_6 substituted heteroaryl;

R_3 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl, C_7 to C_{12} substituted alkylphenyl, naphthyl, substituted naphthyl, C_1 to C_8 alkanesulfonyl, benzenesulfonyl, substituted benzenesulfonyl, or C_1 to C_8 substituted acyl; and

R_4 is:



3. The compound of claim 2, wherein:

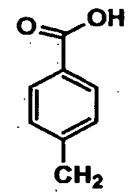
R_1 is C_1 to C_7 alkyl, C_1 to C_7 substituted alkyl, phenyl, substituted phenyl, alkylphenyl, substituted alkylphenyl, C_2 to C_7 heterocyclic ring, C_2 to C_7 substituted heterocyclic ring, C_5 to C_6 heteroaryl, or C_5 to C_6 substituted heteroaryl;

R_2 is C_1 to C_7 alkyl, C_1 to C_7 substituted alkyl, phenyl, substituted phenyl, alkylphenyl, substituted alkylphenyl, C_2 to C_7 heterocyclic ring, C_2 to C_7 substituted heterocyclic ring, C_5 to C_6 heteroaryl, or C_5 to C_6 substituted heteroaryl;

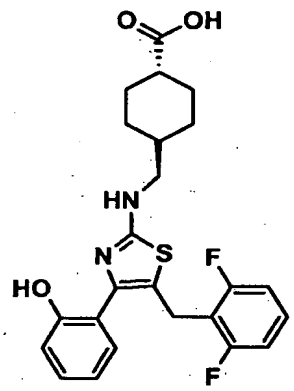
R_3 is hydrogen, C_1 to C_8 alkyl, C_1 to C_8 substituted alkyl, C_7 to C_{12} alkylphenyl, C_7 to C_{12} substituted alkylphenyl, naphthyl, substituted naphthyl, C_1 to C_8

alkanesulfonyl, benzenesulfonyl, substituted benzenesulfonyl, or C_1 to C_8 substituted acyl; and

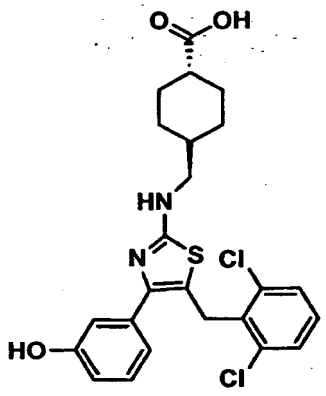
R_4 is:



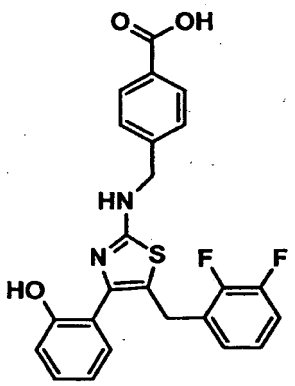
4. The compound according to claim 1, wherein the compound is:



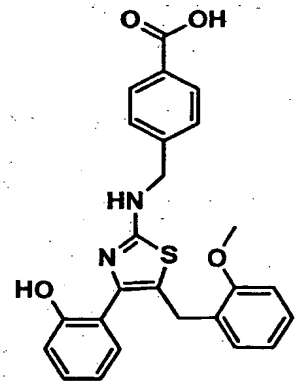
5. The compound according to claim 1, wherein the compound is:



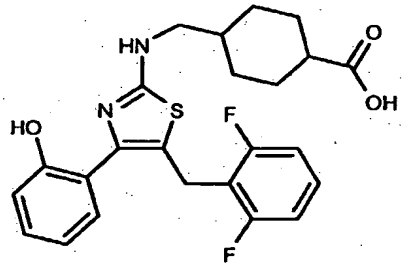
6. The compound according to claim 1, wherein the compound is:



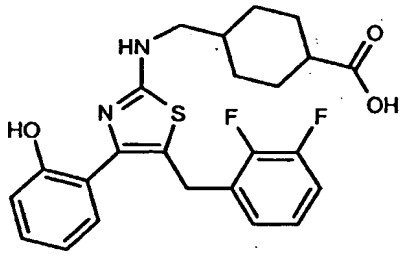
7. The compound according to claim 1, wherein the compound is:



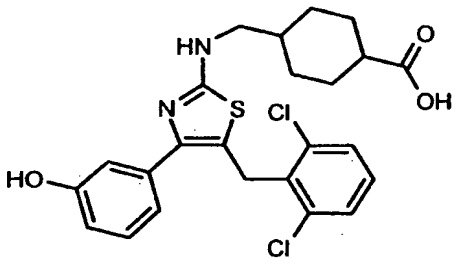
8. The compound according to claim 1, wherein the compound is:



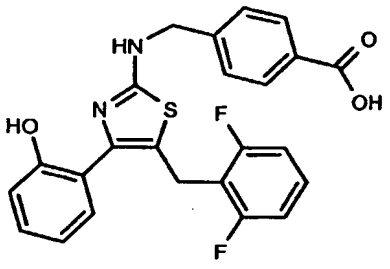
9. The compound according to claim 1, wherein the compound has the following formula:



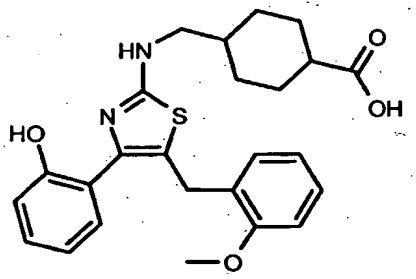
10. The compound according to claim 1, wherein the compound has the following formula:



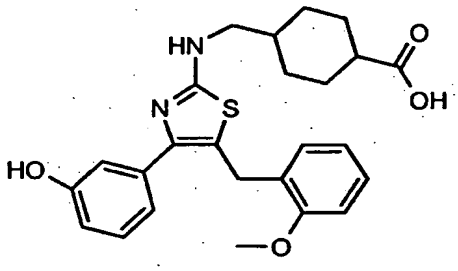
11. The compound according to claim 1, wherein the compound has the following formula:



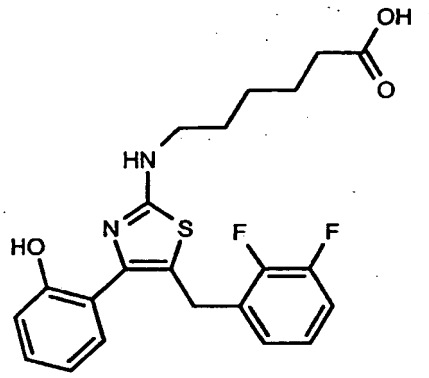
12. The compound according to claim 1, wherein the compound has the following formula:



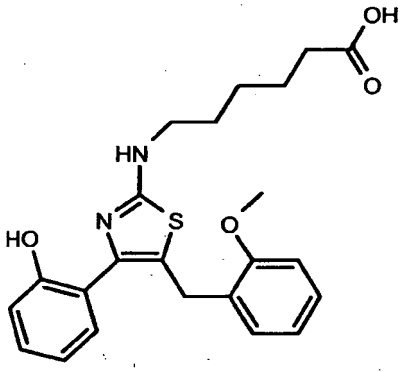
13. The compound according to claim 1, wherein the compound has the following formula:



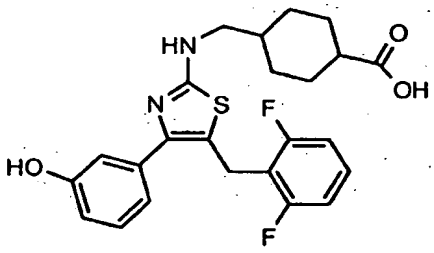
14. The compound according to claim 1, wherein the compound has the following formula:



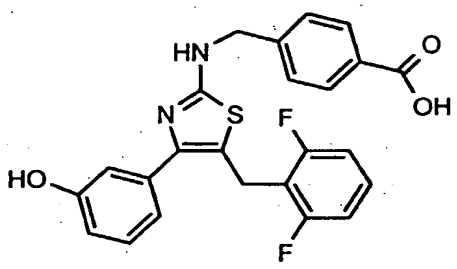
15. The compound according to claim 1, wherein the compound has the following formula:



16. The compound according to claim 1, wherein the compound has the following formula:



17. The compound according to claim 1, wherein the compound has the following formula:



18. A compound of any one of claims 1 to 17 wherein said compound is capable of binding the NR1H4 receptor protein or a portion thereof according to SEQ ID NO. 1 or a mammalian homologue thereof.

19. A therapeutic composition comprising at least one compound according to any one of claims 1 to 17 in admixture with a pharmaceutically acceptable carrier, adjuvant or vehicle.

20. A method for prevention or treatment of a NR1H4 receptor protein- or NR1H4 receptor protein homologue-mediated disease or condition in a mammal comprising administration of a therapeutically effective amount of a compound or combination of compounds according to any one of claims 1 to 17 wherein the prevention or treatment is directly or indirectly accomplished through the binding of the compound to the NR1H4 receptor protein or to the NR1H4 receptor protein homologue.

21. The method for prevention or treatment of a NR1H4 receptor protein mediated disease or condition according to claim 20 wherein the mammal is a human.

22. A method for regulating bile transport in a mammal which comprises activating the NR1H4 receptor with a therapeutically effective amount of a compound according to any one claims 1 to 17.

23. A method of treating a disease which is affected by cholesterol, triglyceride, or bile acid levels comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound or combination of compounds according to any one of claims 1 to 17.

24. A method of treating atherosclerosis, gallstone disease, lipid disorders, obesity or a cardiovascular disorder comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound or combination of compounds according to any one of claims 1 to 17.

25. A method of blocking fatty acid absorption in the intestine of a mammal in need of such blocking comprising administering to the mammal a therapeutically effective amount of a compound or combination of compounds according to any one claims 1 to 17.

26. The method of claim 25, wherein the method is for treating obesity in humans.

27. A method of modulating a gene whose expression is regulated by the NR1H4 receptor in a mammal comprising administering to the mammal a therapeutically effective amount of a compound or combination of compounds according any one of claims 1 to 17.

28. The method according to claim 27 wherein the expression of the gene encoding cholesterol 7-alpha-hydroxylase is lowered.

29. The method according to claim 27 wherein the expression of the gene encoding organic anion protein-1 is lowered.

30. A method according to claim 27 wherein the expression of the gene encoding cholesterol 7-alpha-hydroxylase and organic anion protein-1 are lowered.

31. A method according to claim 27 wherein the expression of the gene encoding intestinal bile acid binding protein is enhanced.

32. A method according to claim 27 wherein the expression the activity of the canicular bile salt excretion pump is enhanced.

33. A method according to claim 27 wherein the expression of the gene encoding intestinal bile acid binding protein and the activity of the canicular bile salt excretion pump are enhanced.

FIG. 1A

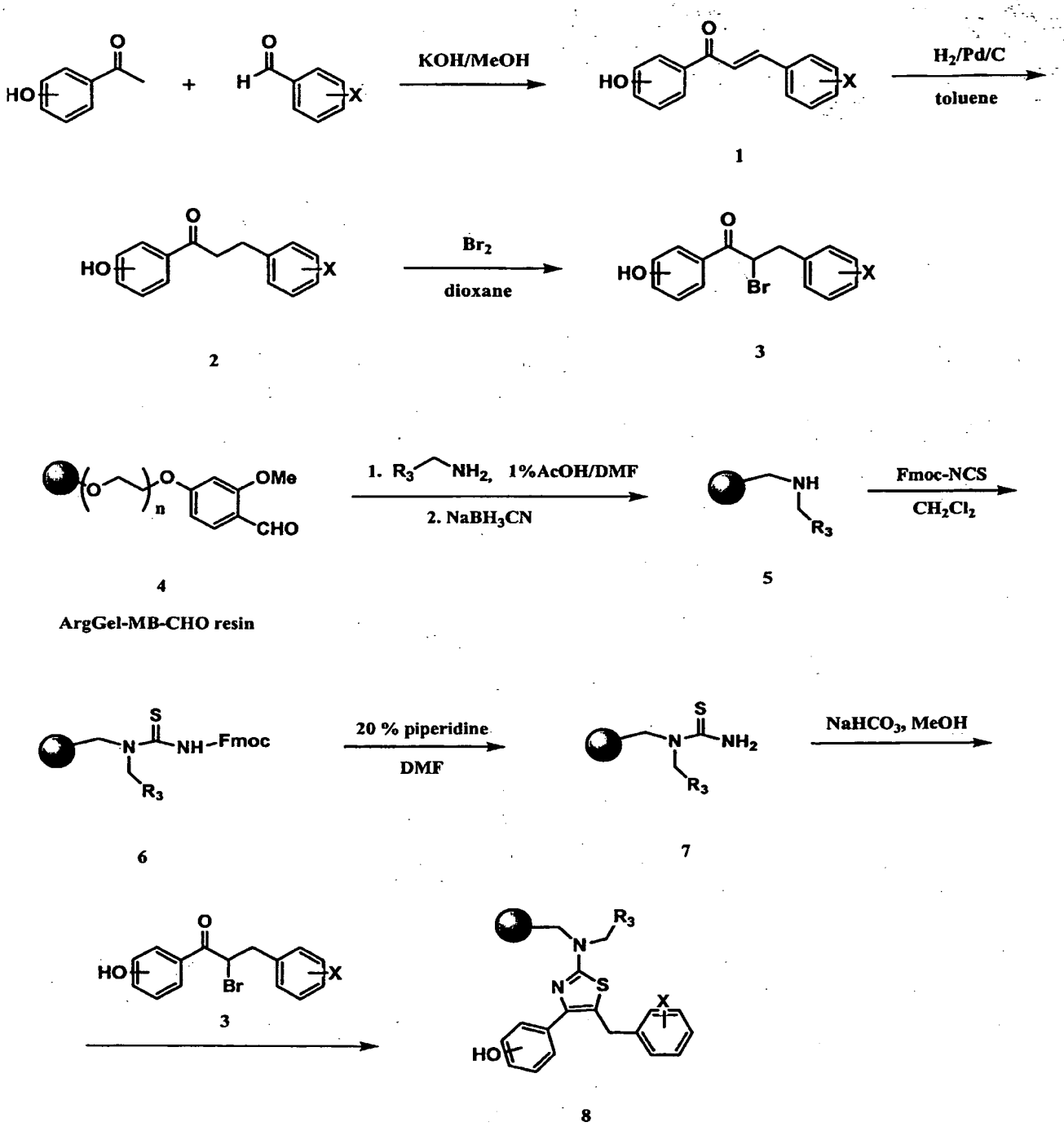
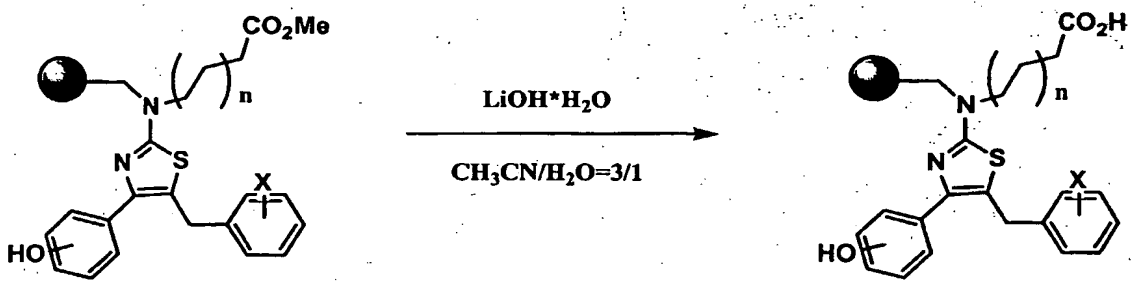
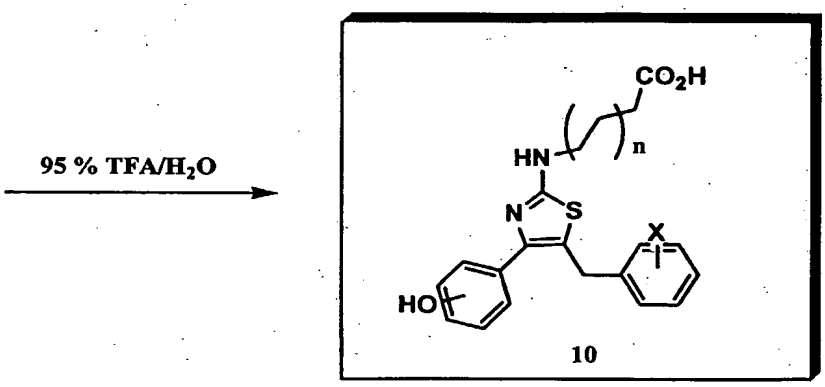

KOH/MeOH
H2/Pd/C
toluene
Br2
dioxane
1. R3 NH2, 1%AcOH/DMF
2. NaBH3CN
Fmoc-NCS
CH2Cl2
20 % piperidine
DMF
NaHCO3, MeOH
1
2
3
4
ArgGel-MB-CHO resin
5
6
7
8

FIG. 1B


CO2Me
n
N
S
X
HO
LiOH*H2O
CH3CN/H2O=3/1
CO2H
n
N
S
X
HO

8 9


95 % TFA/H2O
CO2H
n
HN
N
S
X
HO

10

FIG. 2

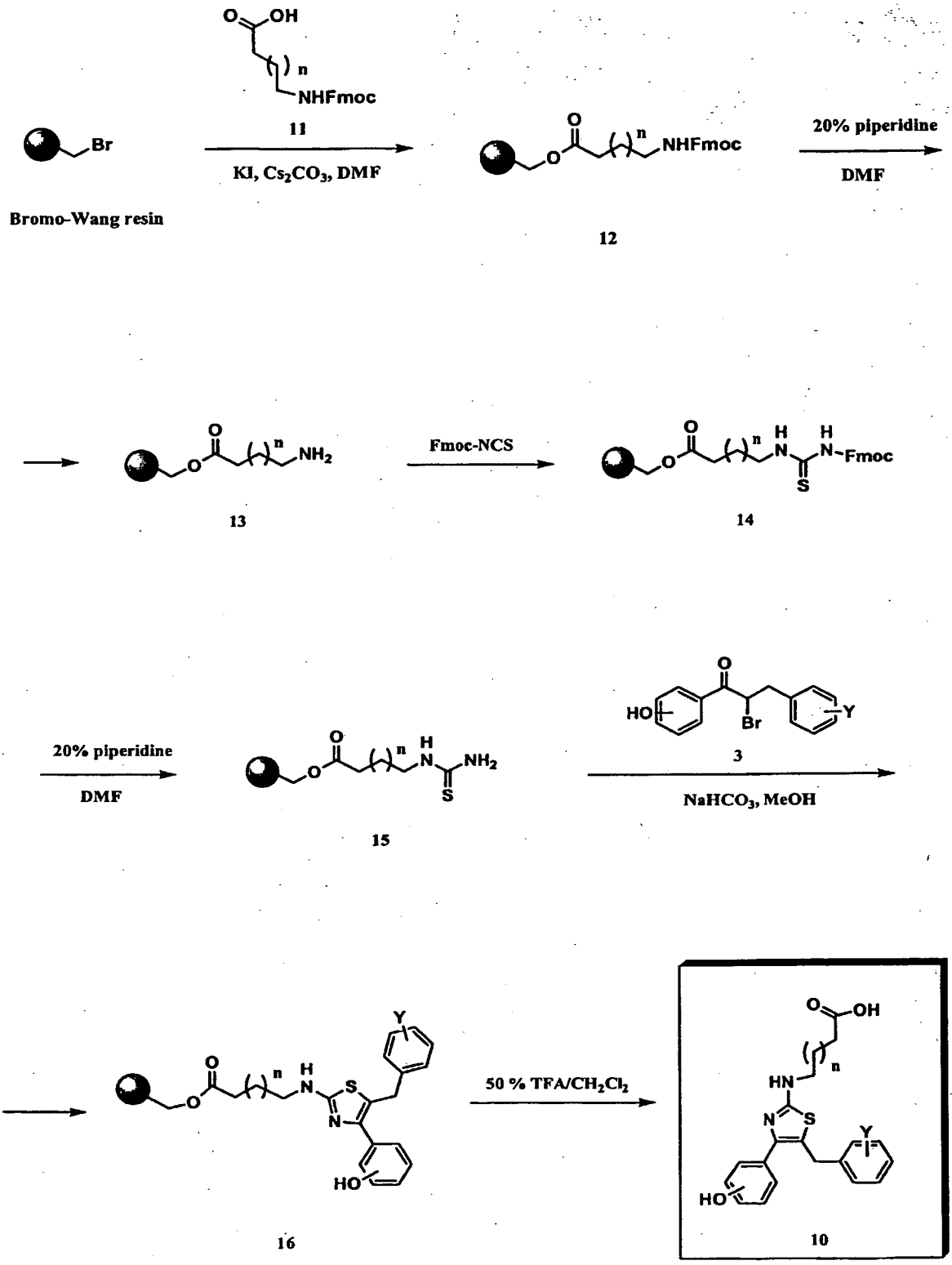

O
OH
n
NHFmoc
11
Br
KI, Cs_2CO_3, DMF
NHFmoc
20% piperidine
DMF
Bromo-Wang resin
12
NH2
Fmoc-NCS
H
N
N-Fmoc
S
13
14
20% piperidine
DMF
HO
Br
Y
3
$NaHCO_3$, MeOH
15
50 % TFA/CH_2Cl_2
HN
S
16
10

FIG. 3

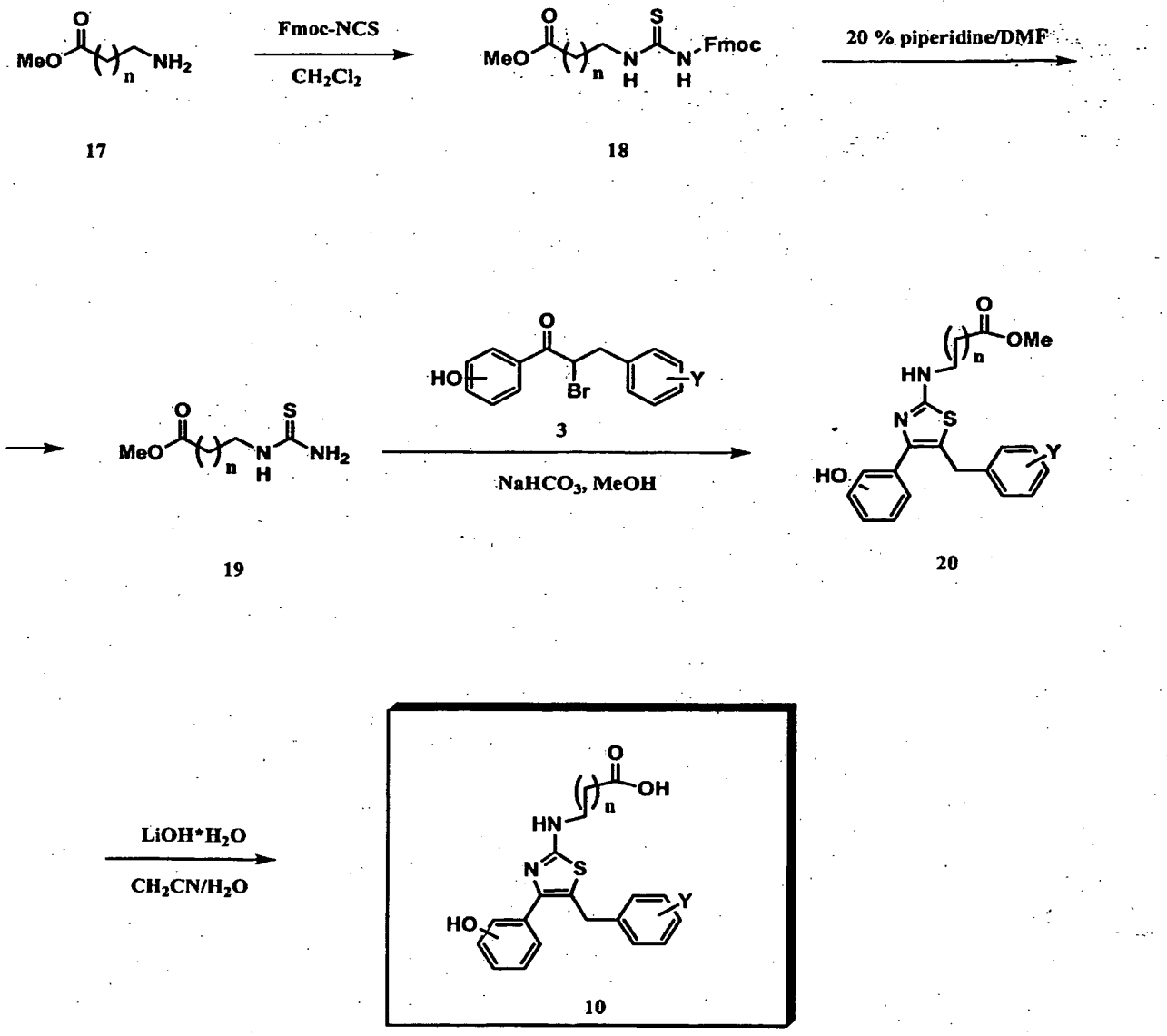

MeO
NH2
n
Fmoc-NCS
CH2Cl2
Fmoc
20 % piperidine/DMF
17
18
HO
Br
Y
3
NaHCO3, MeOH
OMe
HN
N
S
19
20
LiOH*H2O
CH2CN/H2O
OH
10

FIG. 4A

SEQ. ID NO. 1

```
MGSKMNLIEH SHLPTTDEFS FSENLFGVLT EQVAGPLGQN LEVEPYSQYS NVQFPQVQPQ     60
ISSSSYYSNL GFYPQQPEEW YSPGIYELRR MPAETLYQGE TEVAEMPVTK KPRMGASAGR    120
IKGDELCVVC GDRASGYHYN ALTCEGCKGF FRRSITKNAV YKCKNGGNCV MDMYMRRKCQ    180
ECRLRKCKEM GMLAECMYTG LLTEIQCKSK RLRKNVKQHA DQTVNEDSEG RDLRQVTSTT    240
KSCREKTELT PDQQTLLHFI MDSYNKQRMP QEITNKILKE EFSAEENFLI LTEMATNHVQ    300
VLVEFTKKLP GFQTLDHEDQ IALLKGSAVE AMFLRSAEIF NKKLPSGHSD LLEERIRNSG    360
ISDEYITPMF SFYKSIGELK MTQEEYALLT AIVILSPDRQ YIKDREAVEK LQEPLLDVLQ    420
KLCKIHQPEN PQHFACLLGR LTELRTFNHH HAEMLMSWRV NDHKFTPLLC EIWDVQ        476
```

FIG. 4B

SEQ. ID NO. 2

```
atgggatcaa aaatgaatct cattgaacat tcccatttac ctaccacaga tgaatttttct      60
ttttctgaaa atttatttgg tgttttaaca gaacaagtgg caggtcctct gggacagaac      120
ctggaagtgg aaccatactc gcaatacagc aatgttcagt ttccccaagt tcaaccacag      180
atttcctcgt catcctatta ttccaacctg ggtttctacc cccagcagcc tgaagagtgg      240
tactctcctg gaatatatga actcaggcgt atgccagctg agactctcta ccagggagaa      300
actgaggtag cagagatgcc tgtaacaaag aagccccgca tgggcgcgtc agcagggagg      360
atcaaagggg atgagctgtg tgttgtttgt ggagacagag cctctggata ccactataat      420
gcactgacct gtgaggggtg taaaggtttc ttcaggagaa gcattaccaa aaacgctgtg      480
tacaagtgta aaaacggggg caactgtgtg atggatatgt acatgcgaag aaagtgtcaa      540
gagtgtcgac taaggaaatg caaagagatg ggaatgttgg ctgaatgtat gtatacaggc      600
ttgttaactg aaattcagtg taaatctaag cgactgagaa aaaatgtgaa gcagcatgca      660
gatcagaccg tgaatgaaga cagtgaaggt cgtgacttgc gacaagtgac ctcgacaaca      720
aagtcatgca gggagaaaac tgaactcacc ccagatcaac agactcttct acattttatt      780
atggattcat ataacaaaca gaggatgcct caggaaataa caaataaaat tttaaaagaa      840
gaattcagtg cagaagaaaa ttttctcatt ttgacggaaa tggcaaccaa tcatgtacag      900
gttcttgtag aattcacaaa aaagctacca ggatttcaga ctttggacca tgaagaccag      960
attgctttgc tgaaagggtc tgcggttgaa gctatgttcc ttcgttcagc tgagattttc     1020
aataagaaac ttccgtctgg gcattctgac ctattggaag aaagaattcg aaatagtggt     1080
atctctgatg aatatataac acctatgttt agtttttata aaagtattgg ggaactgaaa     1140
atgactcaag aggagtatgc tctgcttaca gcaattgtta tcctgtctcc agatagacaa     1200
tacataaagg atagagaggc agtagagaag cttcaggagc cacttcttga tgtgctacaa     1260
aagttgtgta agattcacca gcctgaaaat cctcaacact ttgcctgtct cctgggtcgc     1320
ctgactgaat tacggacatt caatcatcac cacgctgaga tgctgatgtc atggagagta     1380
aacgaccaca agtttacccc acttctctgt gaaatctggg acgtgcagtg a              1431
```

FIG. 4C

SEQ. ID NO. 3

```
MLVKPLPDSE EEGHDNQEAH QKYETMQCFA VSQPKSIKEE GEDLQSCLIC VARRVPMKER     60
PVLPSSESFT TRQDLQGKIT SLDTSTMRAA MKPGWEDLVR RCIQKFHAQH EGESVSYAKR    120
HHHEVLRQGL AFSQIYRFSL SDGTLVAAQT KSKLIRSQTT NEPQLVISLH MLHREQNVCV    180
MNPDLTGQTM GKPLNPISSN SPAHQALCSG NPGQDMTLSS NINFPINGPK EQMGMPMGRF    240
GGSGGMNHVS GMQATTPQGS NYALKMNSPS QSSPGMNPGQ PTSMLSPRHR MSPGVAGSPR    300
IPPSQFSPAG SLHSPVGVCS STGNSHSYTN SSLNALQALS EGHGVSLGSS LASPDLKMGN    360
LQNSPVNMNP PPLSKMGSLD SKDCFGLYGE PSEGTTGQAE SSCHPGEQKE TNDPNLPPAV    420
SSERADGQSR LHDSKGQTKL LQLLTTKSDQ MEPSPLASSL SDTNKDSTGS LPGSGSTHGT    480
SLKEKHKILH RLLQDSSSPV DLAKLTAEAT GKDLSQESSS TAPGSEVTIK QEPVSPKKKE    540
NALLRYLLDK DDTKDIGLPE ITPKLERLDS KTDPASNTKL IAMKTEKEEM SFEPGDQPGS    600
ELDNLEEILD DLQNSQLPQL FPDTRPGAPA GSVDKQAIIN DLMQLTAENS PVTPVGAQKT    660
ALRISQSTFN NPRPGQLGRL LPNQNLPLDI TLQSPTGAGP FPPIRNSSPY SVIPQPGMMG    720
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RNPAASIPMR PSSQPGQRQT LQSQVMNIGP SELEMNMGGP QYSQQQAPPN QTAPWPESIL    840
PIDQASFASQ NRQPFGSSPD DLLCPHPAAE SPSDEGALLD QLYLALRNFD GLEEIDRALG    900
IPELVSQSQA VDPEQFSSQD SNIMLEQKAP VFPQQYASQA QMAQGSYSPM QDPNFHTMGQ    960
RPSYATLRMQ PRPGLRPTGL VQNQPNQLRL QLQHRLQAQQ NRQPLMNQIS NVSNVNLTLR   1020
PGVPTQAPIN AQMLAQRQRE ILNQHLRQRQ MHQQQQVQQR TLMMRGQGLN MTPSMVAPSG   1080
MPATMSNPRI PQANAQQFPF PPNYGISQQP DPGFTGATTP QSPLMSPRMA HTQSPMMQQS   1140
QANPAYQAPS DINGWAQGNM GGNSMFSQQS PPHFGQQANT SMYSNNMNIN VSMATNTGGM   1200
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KYC                                                                 1263
```

FIG. 4D

SEQ. ID NO. 4

```
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 241 ggacccagcc ccaaaaggaa cactgaaaaa cgtaatcgtg aacaggaaaa taaatatata
 301 gaagaacttg cagagttgat ttttgcaaat tttaatgata tagacaactt taacttcaaa
 361 cctgacaaat gtgcaatctt aaaagaaact gtgaagcaaa ttcgtcagat caaagaacaa
 421 gagaaagcag cagctgccaa catagatgaa gtgcagaagt cagatgtatc ctctacaggg
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 541 ttctttgtag tgaacctgga aggcaacgtt gtgtttgtgt cagagaatgt gacacagtat
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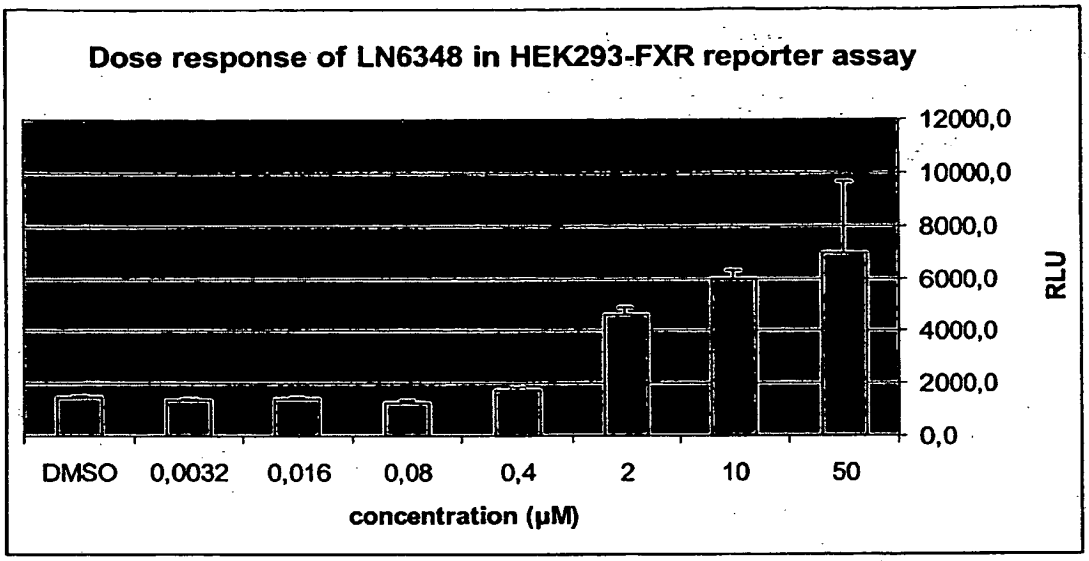
FIG 5 A


Dose response of LN6348 in HEK293-FXR reporter assay
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10000,0
8000,0
6000,0
4000,0
2000,0
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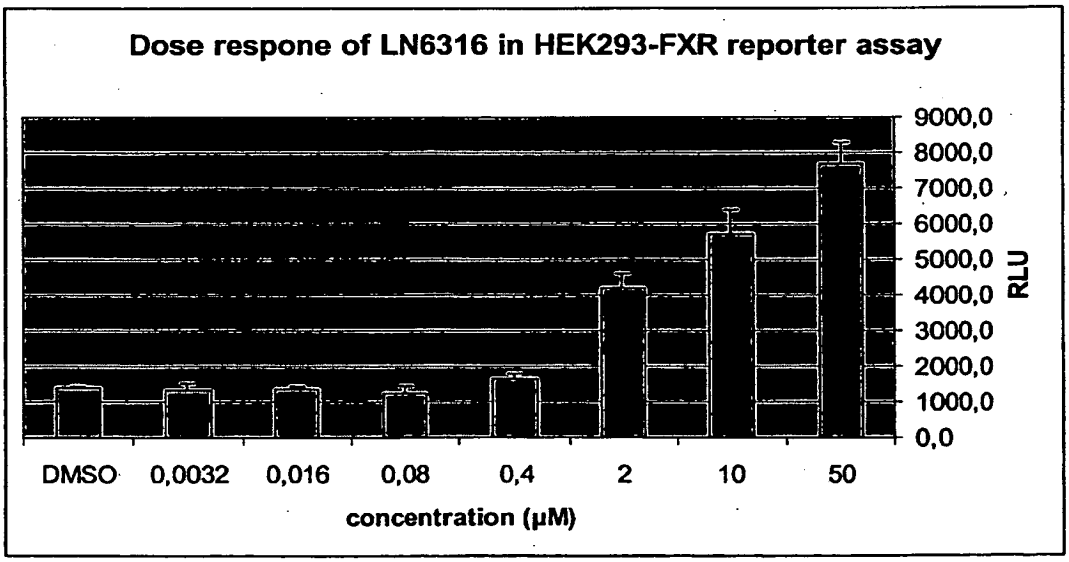
RLU
DMSO
0,0032
0,016
0,08
0,4
2
10
50
concentration (µM)

FIG 5 B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7000,0
6000,0
5000,0
4000,0
3000,0
2000,0
1000,0
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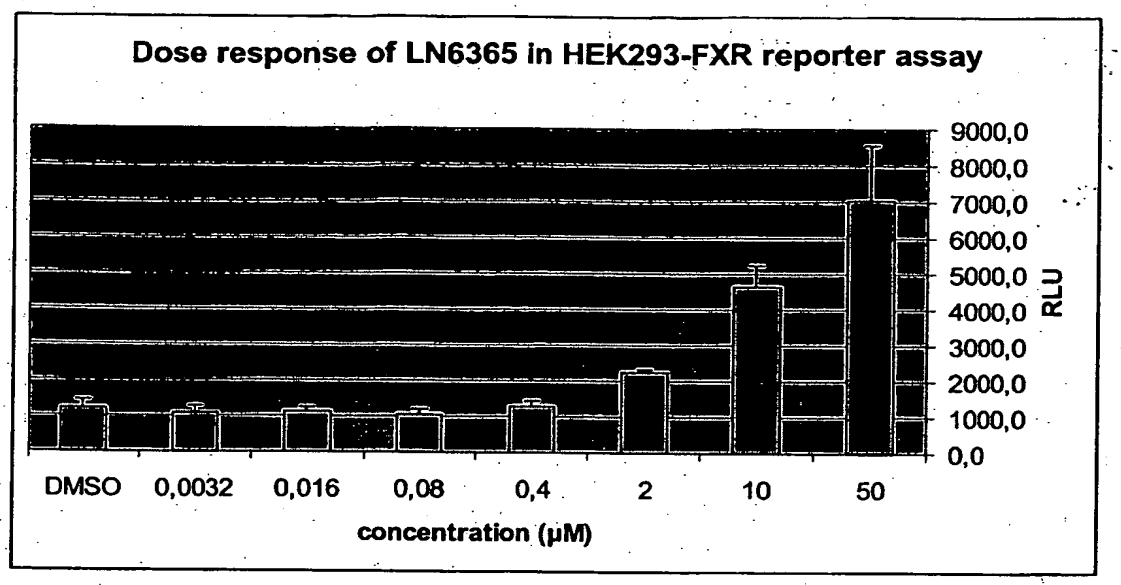
RLU
DMSO
0,0032
0,016
0,08
0,4
2
10
50
concentration (µM)

Fig 5 C


Dose response of LN6365 in HEK293-FXR reporter assay
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4000,0
3000,0
2000,0
1000,0
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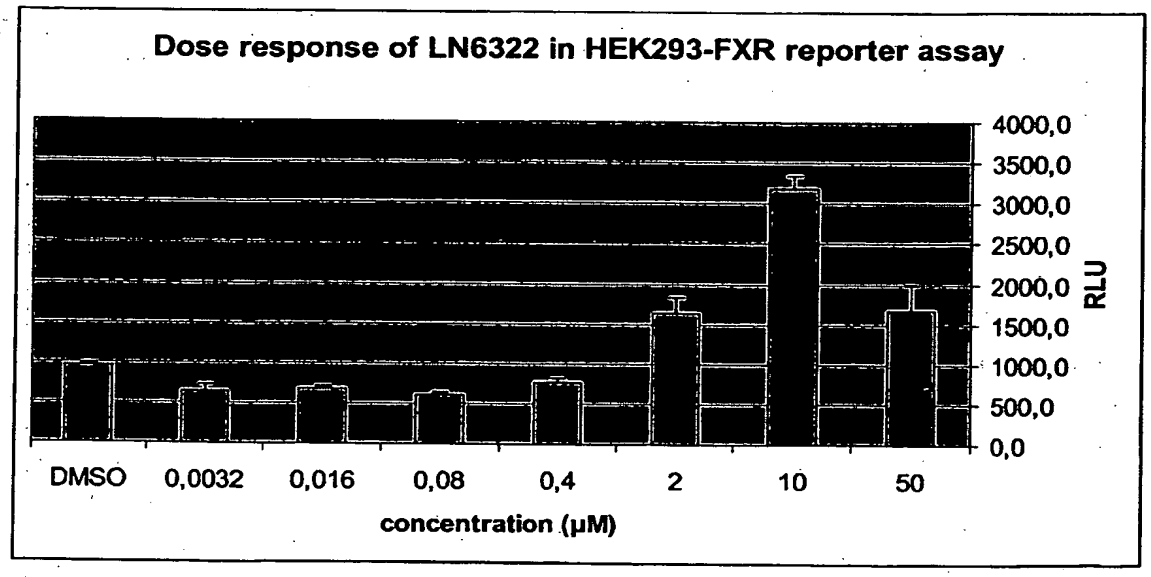
RLU
DMSO
0,0032
0,016
0,08
0,4
2
10
50
concentration (µM)

Fig 5 D


Dose response of LN6322 in HEK293-FXR reporter assay
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RLU
DMSO
0,0032
0,016
0,08
0,4
2
10
50
concentration (µM)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/25458

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/426; C07D 277/40, 277/42, 277/44, 277/46
US CL :514/370; 548/194, 190

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/370; 548/194, 190

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,856,347 A (HASHIGUCHI ET AL.) 05 January 1999 (05/01/99), see entire document, especially columns 1-2 and compounds 1, 76, 112, 113, 171, etc. in Table 1.	1, 3, 6, 7, 11, 14, 15, 17-19
X	JP 09-235278 A (TOYAMA CHEMICAL CO.,) 09 September 1997 (09/09/97), see entire document, especially page 2 and compounds 46 and 72.	1, 18, 19
X	US 5,602,132 A (ROGER ET AL.) 11 February 1997 (11/02/97), see entire document, especially columns 2 and 3; and compounds in columns 45, 46, etc.	1, 18-21, 24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 SEPTEMBER 2002	Date of mailing of the international search report 16 DEC 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LAURA L. STOCKTON Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/25438

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,464,847 A (COURTEMANCHE ET AL.) 07 November 1995 (07/11/95), see entire document, especially columns 2-3; and compounds in columns 45, 46, etc.	1, 18-21, 24